Banana resistance genes and uses thereof

FIELD OF THE INVENTION

[0001] THIS INVENTION relates generally to pathogenic resistance. More particularly, the present invention relates to polynucleotide and polypeptide sequences involved in the resistance mechanism of plants to pathogens, especially fungal pathogens. The present invention also relates to the use of these sequences for modulating plant resistance and for producing genetically modified plants having modified pathogen resistance characteristics.

[0002] Bibliographic details of certain publications referred to by author in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

[0003] Banana is one of the world's most important fruit crops with a world production of approximately 98 million tonnes annually (FAO, 2001). However, as with many monocultures, a range of fungal, viral, bacterial and nematode diseases affects banana, which cause severe economical losses every year.

[0004] Fusarium wilt is one of the most destructive and notorious diseases of banana. It is also known as Panama disease, in recognition of the extensive damage it caused in export plantations in this Central American country. By 1960, Fusarium wilt had destroyed an estimated 40,000 ha of 'Gros Michel' (AAA), causing the export industry to convert to cultivars in the Cavendish subgroup (AAA) (Ploetz and Pegg, 2000). Fusarium wilt is caused by the soilborne hyphomycete, *Fusarium oxysporum* Schlect. f. sp. *cubense*. It is one of more than 120 formae speciales (special forms) of *F. oxysporum* that cause vascular wilts of flowering plants. This pathogen affects species of *Musa* and *Heliconia*, and strains have been classified into four physiological races based on pathogenicity to host cultivars in the field (race 1, 'Gros Michel'; race 2, 'Bluggoe'; race 3, *Heliconia* spp.; and race 4, Cavendish cultivars and all cultivars susceptible to race 1 and 2). Until recently, race 4 had only been recorded to cause serious losses in the subtropical regions of Australia, South Africa, the Canary Islands, and Taiwan. If this race were to become established in the Americas, the world export industries would be severely affected, as there is no widely accepted replacement for Cavendish cultivars (Bentley *et al.*, 1998).

[0005] In general, effective chemical control measures do not exist. In work conducted in South Africa, methyl bromide significantly reduced disease incidence, but was effective for only three years due to recolonisation of the fumigated areas by the pathogen. Studies on the biological and cultural control of this disease have begun only recently. *Arbuscular mycorrhizal* fungi have been shown to

reduce disease severity in short-term green house studies, but results from long term field studies have not been reported (Ortiz *et al.*, 1995). Tissue-culture plantlets are free of pathogens and should be used to establish new plantings whenever possible. However the expense of plantlets may make their use in subsistence agriculture impractical. Genetic resistance offers the greatest opportunity for managing this disease in infested soils (Ortiz *et al.*, 1995).

[0006] Plants recognise and resist many invading pathogens by inducing a rapid defence response, termed the hypersensitive response (HR). The HR results in localised cell and tissue death at the site of infection, which constrains further spread of the infection. This local response often triggers non-specific resistance throughout the plant, a phenomenon known as systemic acquired resistance (SAR). Once triggered, SAR provides resistance to a wide range of pathogens for days. The HR and SAR depend on interaction between a dominant or semidominant resistance gene (R) product in the plant and a corresponding dominant phytopathogen avirulence gene (Avr) product (Baker *et al.*, 1997). A loss or alteration to either the plant R gene or the pathogen Avr gene leads to disease (compatibility) (Hammond-Kosack and Jones, 1997).

[0007] The R proteins provide resistance to pathogens as diverse as fungi, bacteria, viruses, nematodes and insects. Eight classes of R genes have been defined according to the structural characteristics of their predicted protein: (1) cytoplasmic toxin reductase enzymes; (2) intracellular protein kinases; (3) receptor kinase-like protein with two tandem protein kinase domain; (4) receptor-like protein kinases with an extracellular leucine-rich repeat (LRR) domain; (5) intracellular LRR proteins with a nucleotide binding site (NBS) and leucine zipper (LZ) motif; (6) intracellular NBS-LRR proteins with a region with similarity to the Toll and interleukin-1 receptor (TIR) proteins; (7) LRR proteins that encode membrane-bound extracellular proteins; and (8) LZ proteins that encode membrane-bound intracellular proteins (Figure 1). With a few exceptions, all R genes have been cloned by a map-based cloning approach.

[0008] The NBS-LRR class is by far the largest group of resistance proteins with more than 30 cloned genes to date. Two subgroups within the NBS-LRR class have been recognised by the presence or absence of an amino N-terminal region (TIR domain) with amino acid sequence similarity to the cytoplasmic signalling domains of the Toll and interleukin-1 receptors (Meyer *et al.*, 1999; Pan *et al.*, 2000).

[0009] The N-terminal of some NBS-LRR proteins is similar to the cytoplasmic effector domain of the *Drosophila melanogaster* and human TOLL and interleukin-1 receptors (the TIR domain)(Hammond-kosack and Jones, 1997). Other NBS-LRR proteins have different N-terminal domains, which often contain putative leucine-zipper (LZ) motifs. Mutational analysis in *Arabidopsis* revealed that TIR-NBS-LRR and LZ-NBS-LRR proteins employ different signalling pathways. Proteins in the TIR effector domain signal *via* a pathway that includes EDS1, a predicted lipase,

whereas most LZ-NBS-LRR proteins examined employ the membrane-associated NDR1 protein (Aarts *et al.*, 1998). There is no apparent correlation between pathogen type and the NBS-LRR subclass used by plants to detect these pathogens (Ellis and Jones 1998). All this evidence is consistent with the hypothesis of Aarts *et al.*, (1998), who suggested that there may be two downstream pathways triggered by R genes, with the structure of the R protein determining which downstream factors are required. Other recent results have shown that the situation may not be this simple. Two R genes from *Arabidopsis*, RPP8 and RPP13 (both LZ-NBS-LRR proteins), require neither EDS1 nor NDR1, suggesting that there is at least a third pathway for the transduction of R-gene signals (Glazebrook, 2001). Although many studies on different R genes have suggested that the R-protein LRR domain makes the major contribution to the unique recognition capacity of individual R genes, recent analyses of the L allelic series has shown that the TIR domain can also contribute to this capacity. Thus, it is possible that the LRR are necessary but not sufficient for the specific recognition of Avr proteins and that LRR and amino-terminal domains have co-evolved to function in a coordinate manner. (Zachary, 2001).

[0010] The central NBS domain comprises three motifs predicted to bind ATP or GTP, and several conserved motifs whose functions are not known (Hammond-Kosack and Jones, 1997). This region has homology to two activators of apoptosis in animal cells: APAF-1 and CED. By analogy to these well-characterised regulators of programmed cell death, the corresponding domain in NBS-LRR proteins might operate as an intramolecular signal transducer (Van der Biezen and Jones, 1998; Aravind *et al.*, 1999). Domain swaps involving several flax L alleles reveal a requirement for intramolecular interactions and, thus, NBS-LRR proteins might serve as adaptor molecules that link recognition and signal delivery. For example, Avr signals perceived by the LRR might initiate nucleotide hydrolysis at the NBS domain. This might provide the energy necessary for a conformational change in the NBS-LRR protein, exposing its N-terminal effector portion, to trigger a defence response (Van der Biezen and Jones, 1998).

[0011] LRR domain is thought to be involved in ligand-binding and pathogen recognition. LRR contain leucines or other hydrophobic residues at regular intervals and can also contain regularly spaced prolines and asparagines (Bent, 1996). Comparative analyses of the LRR domain show hypervariability, suggesting diversification due to selection pressures. This indicates that recognition specificity resides in this part of the LRR. By analyses of *in vivo* and *in vitro* generated recombinants between different flax L alleles, Ellis *et al.* (1997) confirmed experimentally that the LRR constitute the principal determinant of specificity for Avr products. Differential specificities of R proteins are often associated with duplications, deletions and sequence exchanges within the regions that encode the LRR. Recently, the LRR-like domain of the rice resistance protein Pita was shown to be required for interaction with Avr-Pita in the yeast two-hybrid system. Furthermore, mutation in either Avr-Pita

Pita that abolished resistance also abolished the interaction *in vitro*. This is the first demonstrated interaction between an LRR-containing R protein and its cognate Avr protein (Jia *et al.*, 2000).

[0012] Some of the resistance genes isolated to date have been transferred to susceptible cultivars of the same species or different species with successful results. For example, the N gene for resistance to Tobacco mosaic virus (TMV) has been transferred to tomato and gives resistance in this species to TMV (Whitham *et al.*, 1996). The Bs2 gene, which encodes *Xanthomonas* resistance in pepper, has been cloned and transferred to tomato, a crop species in which the number of useful resistance genes to this pathogen is limited (Tai *et al.*, 1999). However, the RPS2 gene from *Arabidopsis* is non-functional in transgenic tomato and this phenomenon has been referred to as 'restricted taxonomic functionality' (Tai *et al.*, 1999). These data suggest that there may be difficulties in wide, cross-species resistance-gene transfer, in certain instances, due to R gene specificity (Ellis *et al.*, 2000).

[0013] The ability to isolate and transfer R genes eliminates the issue of retention of unwanted and genetically linked germoplasm, an important problem associated with classical breeding. Although disease-resistance transgenic plants are no yet available commercially, future product development seems likely as our current level of understanding of pathogenesis and plant defence improves (Stuiver and Custers 2002).

[0014] Despite the progress in R gene biology, however, no resistance genes have been isolated to date, which can confer resistance to destructive banana diseases in susceptible cultivars.

[0015] In work leading up to the present invention, four genotypes of banana were investigated to identify candidate R genes that would confer resistance to race 4 of *Fusarium oxysporum fsp cubense*. These genotypes were as follows: Cavendish, which is resistant to race 1 but susceptible to race 4; Calcutta 4, which is resistant to race 1 and race 4; three progeny of *Musa acuminata* spp *malaccensis*, which are susceptible to race 4; and three progeny of *Musa acuminata* spp *malaccensis*, which are resistant to race 4. Five families of R genes were identified from this investigation, all of which were present in the genomes of each of the genotypes but which had slightly different sequences. Surprisingly, two of these families (RGA2 and RGA5) were found to share some sequence similarity with the I2 R gene, which confers resistance to Fusarium wilt in tomatoes. In addition RGA2 was shown to be transcribed in the three resistant *Musa acuminata* spp *malaccensis* progeny but not in the three susceptible progeny. These discoveries have been reduced to practice in compositions and methods for modulating disease resistance, especially fungal resistance, in plants including banana and in plants and plant parts, especially genetically modified plants, plant cells, tissues and seeds, having modified disease resistance, as described hereafter.

SUMMARY OF THE INVENTION

[0016] Accordingly, in one aspect, the present invention provides isolated polynucleotides, which in some embodiments, confer disease resistance to a plant, especially resistance to diseases caused by fungal pathogens. These polynucleotides are generally selected from: (a) a polynucleotide comprising a nucleotide sequence that encodes a polypeptide conferring disease resistance to a plant, the sequence sharing at least 30% (and at least 31% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 1 or 3, or a complement thereof; (b) a polynucleotide comprising a portion at least 300 contiguous nucleotides in length of the sequence set forth in SEQ ID NO: 1 or 3 or of a complement of that sequence, wherein the portion encodes a polypeptide that confers disease resistance to a plant; (c) a polynucleotide comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or 4; (d) a polynucleotide comprising a nucleotide sequence that encodes a portion at least 100 contiguous amino acid residues in length of the amino acid sequence set forth in SEQ ID NO: 2 or 4, wherein the portion confers disease resistance to a plant; (e) a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that shares at least 50% (and at least 51% to at least 99% and all integer percentages in between) sequence similarity with at least a portion at least 300 contiguous amino acid residues in length of the sequence set forth in SEQ ID NO: 2 or 4, wherein the polypeptide confers disease resistance to a plant; (f) a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that confers disease resistance to a plant, wherein the polynucleotide hybridises to the sequence of (a), (b), (c), (d), (e) or to a complement thereof, under at least low, medium or high stringency conditions; and (g) a polynucleotide comprising a portion at least 15 contiguous nucleotides in length of the sequence set forth in SEQ ID NO: 1 or 3, or of a complement of that sequence, wherein the portion hybridises to a sequence of (a), (b), (c), (d), (e) or to a complement thereof, under at least low, medium or high stringency conditions.

[0017] In another aspect, the present invention provides nucleic acid constructs for conferring disease resistance to a plant. These constructs generally comprise a polynucleotide as broadly described operably connected to a regulatory element, which is operable in the plant. In certain embodiments, the construct is in the form of a vector, especially an expression vector.

[0018] In yet another aspect, the present invention provides isolated host cells containing a nucleic acid construct as broadly described above. In certain advantageous embodiments, the host cells are plant cells. In some embodiments, the plant cells have the nucleic acid construct incorporated into their nucleome, especially stably incorporated into their genome.

[0019] In still another aspect, the present invention provides plants containing cells comprising a nucleic acid construct as broadly described above. In certain desirable embodiments, the plants have the nucleic acid construct stably incorporated into the nucleome, especially, the genome of their cells.

[0020] In a further aspect, the present invention provides probes for interrogating nucleic acid for the presence of a disease resistance-conferring polynucleotide or portion thereof. These probes generally comprise a nucleotide sequence that hybridises under at least low, medium or high stringency conditions to a polynucleotide as broadly described above. In some embodiments, the probes consist essentially of a nucleic acid sequence which corresponds or is complementary to at least a portion of a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2 or 4, wherein the portion is at least 15 nucleotides in length. In other embodiments, the probes comprise a nucleotide sequence that is capable of hybridising to at least a portion of a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2 or 4 under at least low, medium or high stringency conditions, wherein the portion is at least 15 nucleotides in length. In still other embodiments, the probes comprise a nucleotide sequence that is capable of hybridising to at least a portion of SEQ ID NO: 1 or 3 under at least low, medium or high stringency conditions, wherein the portion is at least 15 nucleotides in length.

[0021] Another aspect of the present invention provides methods for modulating, especially stimulating or enhancing, disease resistance in a plant. These methods generally comprise introducing a construct as broadly described above into the nucleome of the plant and regenerating stably transformed plants. In some embodiments, the construct is introduced into regenerable plant cells so as to yield transformed plant cells, which are suitably identified and selected, and which are subsequently used for regenerating differentiated plants. Typically, a transformed plant cell line is selected from the transformed plant cells for the differentiation of a genetically modified or transgenic plant. In some embodiments, the regenerable cells are regenerable dicotyledonous plant cells. In other embodiments, the regenerable cells are regenerable monocotyledonous plant cells such as regenerable graminaceous monocotyledonous plant cells and especially regenerable non-graminaceous monocotyledonous plant cells. In one example, the regenerable plant cells are regenerable banana cells. In certain advantageous embodiments, the expression of the polynucleotide that is operably linked to the regulatory element in the nucleic acid construct renders the differentiated transgenic plant with enhanced resistance to disease particularly diseases caused by fungal pathogens, especially soil borne fungi such as *Fusarium* species. Desirably, the nucleic acid construct is transmitted through a complete cycle of the differentiated transgenic plant to its progeny so that it is expressed by the progeny plants. Thus, the invention also provides seed, plant parts, tissue, and progeny plants derived from the differentiated transgenic plant.

[0022] In still another aspect, the invention contemplates conventional plant breeding methods to transfer genetic material corresponding to a polynucleotide as broadly described above *via* crossing and backcrossing to other plants, especially plants that are susceptible to a pathogenic disease, especially a disease caused by fungal pathogens such as species of *Fusarium*. In some embodiments, the genetic material will comprise naturally-occurring DNA that corresponds to a polynucleotide as

broadly described above. Typically, these methods will comprise the steps of: (1) sexually crossing a plant containing that genetic material with a plant from a pathogen susceptible taxon; (2) recovering reproductive material from the progeny of the cross; and (3) growing plants with enhanced resistance to the disease from the reproductive material. In some embodiments, the methods will further comprise prior to step (1): identifying a plant that is resistant to the pathogenic disease by detecting expression in the plant of a polynucleotide as broadly described above. In certain advantageous embodiments, these methods will further comprise the steps of repetitively: (a) backcrossing the disease resistant progeny with disease susceptible plants from the susceptible taxon; and (b) selecting for expression of a nucleic acid sequence corresponding to a polynucleotide as broadly described above (or an associated marker gene) among the progeny of the backcross, until the desired characteristics of the susceptible taxon are present in the progeny along with the gene or genes imparting the pathogen resistance.

[0023] In another aspect of the invention, there is provided isolated polypeptides, which in some embodiments, confer disease resistance to a plant. These polypeptides are generally selected from: (i) a polypeptide comprising an amino acid sequence that shares at least 50% (and at least 51% to at least 99% and all integer percentages in between) similarity with the sequence set forth in SEQ ID NO: 2 or 4; (ii) a polypeptide comprising a portion at least 100 contiguous amino acid residues in length of the sequence set forth in SEQ ID NO: 2 or 4, wherein the polypeptide confers disease resistance to a plant; (iii) a polypeptide comprising an amino acid sequence that shares at least 30% (and at least 31% to at least 99% and all integer percentages in between) similarity with at least a portion of the sequence set forth in SEQ ID NO: 2 or 4, wherein the portion is at least 100 contiguous amino acid residues in length; and (iv) a polypeptide comprising at least a portion of of the sequence set forth in SEQ ID NO: 2 or 4, wherein the portion is at least 5 contiguous amino acid residues in length and is immuno-interactive with an antigen-binding molecule that is immuno-interactive with a sequence selected from (i), (ii) or (iii).

[0024] In some embodiments, the polypeptide includes one or more and in some cases all of the following domains (the numbering refers to the consensus numbering in Figure 2):

a domain which corresponds to residues 1-167 of Figure 2. This domain may be structurally similar to a coiled coil. In some embodiments, this domain can have at least 60, 70, 80, 90, 95, or 98% (and all integer percentages in between) sequence similarity with, or have at least 30, 40, 50, 60, 70 or 80% (and all integer percentages in between) sequence identity to, or differ at no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 or 40 (and all integers in between) amino acid residues from, the corresponding domain of any of the sequences presented in Figure 2;

a domain which corresponds to residues 168-536 of Figure 2. This domain may be functionally analogous to a nuclear-binding site (NBS) domain. In some embodiments, this domain

can have at least 70, 80, 90, 95, or 98% (and all integer percentages in between) sequence similarity with, or have at least 50, 60, 70, 80 or 90% (and all integer percentages in between) sequence identity to, or differ at no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 or 40 (and all integers in between) amino acid residues from, the corresponding domain of any of the sequences presented in Figure 2; and

a domain which corresponds to residues 537-1476 of Figure 2. This domain may be functionally analogous to a leucine-rich repeat (LRR) domain. In some embodiments, this domain can have at least 60, 70, 80, 90, 95, or 98% (and all integer percentages in between) sequence similarity with, or have at least 30, 40, 50, 60, 70, 80 or 90% (and all integer percentages in between) sequence identity to, or differ at no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 or 40 (and all integers in between) amino acid residues from, the corresponding domain of any of the sequences presented in Figure 2.

[0025] In some embodiments, it may be desirable to conserve one or more of the residues in the above regions, which residues are conserved between the sequences presented in Figure 2, wherein the conserved amino acid residues correspond to identical residues or to residues belonging to the same class or subclass of amino acid residues.

[0026] In some embodiments, the domain corresponding to residues 1-167 of Figure 2 comprises a sequence according to Formula (I):

Ser-Φaa_1-Φaa_2 Zaa-Xaa_1-Φaa_3-Φaa_4 Xaa_2-Baa_1 Σaa_1-Xaa_3-Asn-Xaa_4-Xaa_5-Φaa_5-Xaa_6-Xaa_7-Leu-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}-Xaa_{13}-Baa_2-Xaa_{14}-$Åaa_1$-Leu-Xaa_{15}-Xaa_{16}-Leu-Xaa_{17}-Xaa_{18}-Σaa_2-Leu-Leu-Arg-Xaa_{19}-His-Σaa_3-Φaa_6-Leu-$Åaa_2$-Ωaa_1-Ala-Ωaa_2-Σaa_4-Arg-Xaa_{20}-Xaa_{21}-Xaa_{22}-Xaa_{23}-Xaa_{24}-Xaa_{25}-Xaa_{26}-Ser-Leu-Val-Xaa_{27}-Φaa_7-Φaa_8-Xaa_{28}-Xaa_{29}-Leu-Lys-$Åaa_3$-Xaa_{30}-Ala-Tyr-Asp-Ala-$Åaa_4$-Asp-Φaa_9-Leu-$Åaa_5$-Glu-Φaa_{10}-Glu-Xaa_{31}-Xaa_{32}-Ala-Xaa_{33}-Baa_3-Xaa_{34}-Lys-Val (I)

wherein: each of Φ_{1-10} is independently selected from any hydrophobic amino acid residue,
Zaa is a neutral/polar amino acid residue,
each of Σaa_{1-4} is independently selected from any small amino acid residue,
each of Baa_{1-3} is independently selected from any basic amino acid residue,
each of $Åaa_{1-5}$ is independently selected from any acidic amino acid residue,
each of Ωaa_{1-2} is independently selected from any charged amino acid residue, and
Xaa_{1-33} are each independently selected from any amino acid residue.

[0027] In some embodiments, Zaa is selected from Gln or Asn.

[0028] In some embodiments, Φaa_1 is selected from Phe or Leu. In some embodiments, Φaa_2 is selected from Ile or Val. In some embodiments, Φaa_3 is selected from Leu or Ile. In some embodiments, Φaa_4 is selected from Leu or Phe. In some embodiments, Φaa_5 is selected from Ile or Val. In some embodiments, Φaa_6 is selected from Ile or Leu. In some embodiments, Φaa_7 is selected

from Leu or Trp. In some embodiments, Φaa_8 is selected from Val or Leu. In some embodiments, Φaa_9 is selected from Leu or Ile. In some embodiments, Φaa_{10} is selected from Leu or Trp.

[0029] In some embodiments, Σaa_1 is selected from Ala Ser. In some embodiments, Σaa_2 is selected from Ser or Thr. In some embodiments, Σaa_3 is selected from Ala Ser. In some embodiments, Σaa_4 is selected from Thr or Ala.

[0030] In some embodiments, Baa_1 is selected from Lys or Arg. In some embodiments, Baa_2 is selected from His or Arg. In some embodiments, Baa_3 is selected from Lys or Arg.

[0031] In some embodiments, each of $Åaa_{1-5}$ is independently selected from Asp or Glu.

[0032] In some embodiments, Ωaa_1 is selected from Lys or Glu. In some embodiments, Ωaa_2 is selected from Glu or Lys.

[0033] In some embodiments Xaa_1 is a small or acidic amino acid residue, e.g., Xaa_1 is selected from Thr or Glu. In some embodiments, Xaa_2 is an acidic or neutral/polar amino acid residue, e.g., Xaa_2 is selected from Asp or Asn. In some embodiments, Xaa_3 is a small or hydrophobic amino acid residue, e.g., Xaa_3 is selected from Ser or Ile. In some embodiments, Xaa_4 is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_4 is selected from Cys or Leu. In some embodiments, Xaa_5 is a small or hydrophobic amino acid residue, e.g., Xaa_5 is selected from Ala Ile. In some embodiments, Xaa_6 is a neutral/polar or small amino acid residue, e.g., Xaa_6 is selected from Gln or Ala. In some embodiments, Xaa_7 is a neutral/polar or acidic amino acid residue, e.g., Xaa_7 is selected from Gln or Glu. In some embodiments, Xaa_8 is a small or basic amino acid residue, e.g., Xaa_8 is selected from Ala Arg. In some embodiments, Xaa_9 is a basic or hydrophobic amino acid residue, e.g., Xaa_9 is selected from Arg or Leu.

[0034] In some embodiments, Xaa_{10} is a basic or neutral/polar amino acid residue, e.g., Xaa_{10} is selected from Arg or Gln. In some embodiments, Xaa_{11} is a basic or hydrophobic amino acid residue, e.g., Xaa_{11} is selected from Arg or Leu. In some embodiments, Xaa_{12} is a small or neutral/polar amino acid residue, e.g., Xaa_{12} is selected from Arg or Gln. In some embodiments, Xaa_{13} is a hydrophobic or small amino acid residue, e.g., Xaa_{13} is selected from Leu or Ala. In some embodiments, Xaa_{14} is an acid or small amino acid residue, e.g., Xaa_{14} is selected from Asp or Ala. In some embodiments, Xaa_{15} is a basic or neutral/polar amino acid residue, e.g., Xaa_{15} is selected from Arg or Asn. In some embodiments, Xaa_{16} is a basic or neutral/polar amino acid residue, e.g., Xaa_{16} is selected from Arg or Asn. In some embodiments, Xaa_{17} is a basic or neutral/polar amino acid residue, e.g., Xaa_{17} is selected from Arg or Gln. In some embodiments, Xaa_{18} is a small or basic amino acid residue, e.g., Xaa_{18} is selected from Thr or Arg. In some embodiments, Xaa_{19} is a hydrophobic or small amino acid residue, e.g., Xaa_{19} is selected from Ile or Thr.

[0035] In some embodiments, Xaa_{20} is a hydrophobic or basic amino acid residue, e.g., Xaa_{20} is selected from Trp or Arg. In some embodiments, Xaa_{21} is absent or is a neutral/polar amino acid residue, e.g., Asn. In some embodiments, Xaa_{22} is a basic or hydrophobic amino acid residue, e.g., Xaa_{22} is selected from His or Met. In some embodiments, Xaa_{23} is a basic or small amino acid residue, e.g., Xaa_{23} is selected from Lys or Thr. In some embodiments, Xaa_{24} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{24} is selected from Asn or Asp. In some embodiments, Xaa_{25} is a small or basic amino acid residue, e.g., Xaa_{25} is selected from Thr or Lys. In some embodiments, Xaa_{26} is an acidic or hydrophobic amino acid residue, e.g., Xaa_{26} is selected from Glu or Leu. In some embodiments, X_{27} is a basic or hydrophobic or amino acid residue, e.g., Xaa_{27} is selected from Arg or Met. In some embodiments, Xaa_{28} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{28} is selected from Gln or Glu. In some embodiments, Xaa_{29} is a small or hydrophobic amino acid residue, e.g., Xaa_{29} is selected from Ala Trp.

[0036] In some embodiments, Xaa_{30} is a hydrophobic or small amino acid residue, e.g., Xaa_{30} is selected from Tyr or Ala. In some embodiments, Xaa_{31} is a neutral/polar or small amino acid residue, e.g., Xaa_{31} is selected from Gln or Ala. In some embodiments, Xaa_{32} is a small or hydrophobic amino acid residue, e.g., Xaa_{32} is selected from Ala Ile. In some embodiments, Xaa_{33} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{33} is selected from Gln or Leu.

[0037] In some embodiments, the domain corresponding to residues 168-536 of Figure 2 comprises a sequence according to Formula (II):

Arg-Xaa_1-Xaa_2-Thr-Σaa_1-Ser-Φaa_1-Leu-Thr-Glu-Σaa_2-Xaa_3-Φaa_2-Φaa_3-Gly-Arg-Xaa_4-Gln-$Åaa_1$-Baa_1-Glu-Xaa_5-Φaa_4-Φaa_5-Ωaa_1-Leu-Leu-Leu-$Åaa_2$-Σaa_3-Σaa_4-Xaa_6-Gly-Xaa_7-Xaa_8-Σaa_5-Phe-Σaa_6-Val-Φaa_6-Pro-Φaa_7-Val-Gly-Φaa_8-Gly-Gly-Xaa_9-Gly-Lys-Thr-Thr-Leu-Σaa_7-Gln-Leu-Φaa_9-Φaa_{10}-Asn-Asp-Xaa_{10}-Arg-Val-Xaa_{11}-Xaa_{12}-Xaa_{13}-Phe-Xaa_{14}-Leu-Baa_2-Φaa_{11}-Trp-Val-Cys-Val-Ser-Asp-Xaa_{15}-Phe-Xaa_{16}-Val-Lys-Arg-Φaa_{12}-Thr-Baa_3-Glu-Ile-Xaa_{17}-Glu-Xaa_{18}-Ala-Thr-Xaa_{19}-Xaa_{20}-Ωaa_2-Xaa_{21}-Xaa_{22}-Asp-Xaa_{23}-Xaa_{24}-Asn-Leu-Xaa_{25}-Xaa_{26}-Leu-Gln-Xaa_{27}-Xaa_{28}-Leu-Lys-Glu-Ωaa_3-Ile-Xaa_{29}-Σaa_8-Xaa_{30}-Xaa_{31}-Phe-Leu-Leu-Val-Leu-Asp-Asp-Val-Trp-Xaa_{32}-Glu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Ωaa_4-Trp-Glu-Xaa_{36}-Leu-Xaa_{37}-Ala-Pro-Leu-Ωaa_5-Xaa_{38}-Σaa_9-Σaa_{10}-Arg-Gly-Ser-Xaa_{39}-Val-Ile-Val-Thr-Thr-Xaa_{40}-Xaa_{41}-Xaa_{42}-Lys-Φaa_{13}-Ala-Xaa_{43}-Φaa_{14}-Xaa_{44}-Gly-Thr-Met-Ωaa_6-Xaa_{45}-Φaa_{15}-Xaa_{46}-Leu-$Åaa_3$-Xaa_{47}-Leu-Xaa_{48}-$Åaa_4$-Asp-Xaa_{49}-Xaa_{50}-Trp-Xaa_{51}-Leu-Φaa_{16}-Ωaa_7-Xaa_{52}-Xaa_{53}-Σaa_{11}-Phe-Xaa_{54}-Xaa_{55}-Xaa_{56}-Xaa_{57}-Xaa_{58}-Σaa_{12}-Xaa_{59}-Xaa_{60}-Xaa_{61}-Xaa_{62}-Ωaa_8-Φaa_{17}-Glu-Xaa_{63}-Ile-Gly-Arg-Lys-Ile-Ala-Xaa_{64}-Lys-Φaa_{18}-Xaa_{65}-Gly-Xaa_{66}-Pro-Φaa_{19}-Σaa_{13}-Ala-Xaa_{67}-Σaa_{14}-Φaa_{20}-Gly-Xaa_{68}-Φaa_{21}-Leu-Arg-Xaa_{69}-Ωaa_9-Xaa_{70}-Σaa_{15}-Xaa_{71}-Xaa_{72}-Xaa_{73}-Trp-Arg-Xaa_{74}-Φaa_{22}-Φaa_{23}-Glu-Σaa_{16}-Glu-Xaa_{75}-Trp-Xaa_{76}-Φaa_{24}-Pro-Xaa_{77}-Ala-Xaa_{78}-Xaa_{79}-$Åaa_5$-Φaa_{25}-Leu-Σaa_{17}-Xaa_{80}-Leu-Xaa_{81}-Xaa_{82}-Ser-Tyr-Xaa_{83}-Xaa_{84}-Leu-Pro-Σaa_{18}-Xaa_{85}-Leu-Baa_4-Xaa_{86}-Cys-Phe-Ala-Phe-Cys-Ala-Φaa_{26}-Phe-

Xaa_{87}-Lys-Xaa_{88}-Tyr-Xaa_{89}-Phe-Xaa_{90}-Lys-Ωaa_{10}-Xaa_{91}-Leu-Ile-Xaa_{92}-Xaa_{93}-Trp-Ile-Ala-Xaa_{94}-Xaa_{95}-Φaa_{27}-Ile (II)

wherein: each of Φ_{1-27} is independently selected from any hydrophobic amino acid residue,
each of Σaa_{1-18} is independently selected from any small amino acid residue,
each of Baa_{1-4} is independently selected from any basic amino acid residue,
each of $Åaa_{1-5}$ is independently selected from any acidic amino acid residue,
each of Ωaa_{1-10} is independently selected from any charged amino acid residue, and
Xaa_{1-95} are each independently selected from any amino acid residue.

[0038] In some embodiments, Σaa_1 is selected from Ser or Thr. In some embodiments, Σaa_2 is selected from Thr or Ser. In some embodiments, Σaa_3 is selected from Ser or Pro. In some embodiments, Σaa_4 is selected from Gly or Ser. In some embodiments, Σaa_5 is selected from Ser or Ala. In some embodiments, Σaa_6 is selected from Ser or Pro. In some embodiments, Σaa_7 is selected from Ala or Ser. In some embodiments, Σaa_8 is selected from Ser or Gly. In some embodiments, Σaa_9 is selected from Ala or Gly.

[0039] In some embodiments, Σaa_{10} is selected from Ala or Gly. In some embodiments, Σaa_{11} is selected from Ala or Ser. In some embodiments, Σaa_{12} is selected from Pro or Ser. In some embodiments, Σaa_{13} is selected from Ala or Gly. In some embodiments, Σaa_{14} is selected from Thr or Ala. In some embodiments, Σaa_{15} is selected from Ser or Gly. In some embodiments, Σaa_{16} is selected from Ser or Thr. In some embodiments, Σaa_{17} is selected from Pro or Ser. In some embodiments, Σaa_{18} is selected from Gly or Pro.

[0040] In some embodiments, Φaa_1 is selected from Phe or Leu. In some embodiments, Φaa_2 is selected from Val or Ile. In some embodiments, Φaa_3 is selected from Phe or Val. In some embodiments, Φaa_4 is selected from Val or Leu. In some embodiments, Φaa_5 is selected from Val or Ile. In some embodiments, Φaa_6 is selected from Leu or Val. In some embodiments, Φaa_7 is selected from Leu or Ile. In some embodiments, Φaa_8 is selected from Ile or Val. In some embodiments, Φaa_9 is selected from Val or Ile.

[0041] In some embodiments, Φaa_{10} is selected from Tyr or Phe. In some embodiments, Φaa_{11} is selected from Val or Met. In some embodiments, Φaa_{12} is selected from Leu or Ile. In some embodiments, Φaa_{13} is selected from Ile or Val. In some embodiments, Φaa_{14} is selected from Ile or Val. In some embodiments, Φaa_{15} is selected from Ile or Tyr. In some embodiments, Φaa_{16} is selected from Phe or Ile. In some embodiments, Φaa_{17} is selected from Leu or Met. In some embodiments, Φaa_{18} is selected from Leu or Ile. In some embodiments, Φaa_{19} is selected from Leu or Tyr.

[0042] In some embodiments, Φaa_{20} is selected from Leu or Met. In some embodiments, Φaa_{21} is selected from Leu or Tyr. In some embodiments, Φaa_{22} is selected from Ile or Val. In some embodiments, Φaa_{23} is selected from Met or Leu. In some embodiments, Φaa_{24} is selected from Leu or Met. In some embodiments, Φaa_{25} is selected from Ile or Val. In some embodiments, Φaa_{26} is selected from Val or Leu. In some embodiments, Φaa_{27} is selected from Phe or Leu.

[0043] In some embodiments, Baa_{1-4} are each independently selected from Arg or Lys.

[0044] In some embodiments, each of $Åaa_{1-5}$ is independently selected from Asp or Glu.

[0045] In some embodiments, Ωaa_1 is selected from Glu or Arg. In some embodiments, Ωaa_2 is selected from Glu or Arg. In some embodiments, Ωaa_3 is selected from Lys or Glu. In some embodiments, Ωaa_4 is selected from Asp or Lys. In some embodiments, Ωaa_5 is selected from Arg or Asp. In some embodiments, Ωaa_6 is selected from Lys or Glu. In some embodiments, Ωaa_7 is selected from Lys or Glu. In some embodiments, Ωaa_8 is selected from Glu or Arg. In some embodiments, Ωaa_9 is selected from Asp or Lys. In some embodiments, Ωaa_{10} is selected from His or Asp.

[0046] In some embodiments Xaa_1 is a basic or small amino acid residue, e.g., Xaa_1 is selected from Arg or Gly. In some embodiments, Xaa_2 is an acidic or hydrophobic amino acid residue, e.g., Xaa_2 is selected from Glu or Val. In some embodiments, Xaa_3 is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_3 is selected from Val or Cys. In some embodiments, Xaa_4 is an acidic or small amino acid residue, e.g., Xaa_4 is selected from Asp or Ala. In some embodiments, Xaa_5 is a basic or neutral/polar amino acid residue, e.g., Xaa_5 is selected from Lys or Asn. In some embodiments, Xaa_6 is a small or acidic amino acid residue, e.g., Xaa_6 is selected from Ser or Asp. In some embodiments, Xaa_7 is absent or is a neutral/polar amino acid residue, e.g., Asn. In some embodiments, Xaa_8 is absent or is a small amino acid residue, e.g., Ser. In some embodiments, Xaa_9 is a hydrophobic or small amino acid residue, e.g., Xaa_9 is selected from Val or Ala.

[0047] In some embodiments, Xaa_{10} is a neutral/polar or basic amino acid residue, e.g., Xaa_{10} is selected from Asn or Lys. In some embodiments, Xaa_{11} is a small or acidic amino acid residue, e.g., Xaa_{11} is selected from Gly or Glu. In some embodiments, Xaa_{12} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{12} is selected from Asn or Glu. In some embodiments, Xaa_{13} is a hydrophobic or basic amino acid residue, e.g., Xaa_{13} is selected from Tyr or His. In some embodiments, Xaa_{14} is a basic or small amino acid residue, e.g., Xaa_{14} is selected from His or Pro. In some embodiments, Xaa_{15} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{15} is selected from Asn or Asp. In some embodiments, Xaa_{16} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{16} is selected from Asn or Asp. In some embodiments, Xaa_{17} is a hydrophobic or small amino acid residue, e.g., Xaa_{17} is selected from Ile or Thr. In some embodiments, Xaa_{18} is a small or hydrophobic amino acid residue, e.g.,

Xaa_{18} is selected from Ser or Tyr. In some embodiments, Xaa_{19} is a basic or neutral/polar amino acid residue, e.g., Xaa_{19} is selected from Lys or Asn.

[0048] In some embodiments, Xaa_{20} is a hydrophobic or small amino acid residue, e.g., Xaa_{20} is selected from Val or Gly. In some embodiments, Xaa_{21} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{21} is selected from Gln or Phe. In some embodiments, Xaa_{22} is a small or hydrophobic amino acid residue, e.g., Xaa_{22} is selected from Ser or Met. In some embodiments, Xaa_{23} is a basic or hydrophobic amino acid residue, e.g., Xaa_{23} is selected from Lys or Leu. In some embodiments, Xaa_{24} is a hydrophobic or small amino acid residue, e.g., Xaa_{24} is selected from Leu or Thr. In some embodiments, Xaa_{25} is an acidic or neutral/polar amino acid residue, e.g., Xaa_{25} is selected from Asp or Asn. In some embodiments, Xaa_{26} is a small or hydrophobic amino acid residue, e.g., Xaa_{26} is selected from Thr or Met. In some embodiments, Xaa_{27} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{27} is selected from Gln or Val. In some embodiments, Xaa_{28} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{28} is selected from Ile or Asn. In some embodiments, Xaa_{29} is a small or basic amino acid residue, e.g., Xaa_{29} is selected from Ala or Arg.

[0049] In some embodiments, Xaa_{30} is an acidic or small amino acid residue, e.g., Xaa_{30} is selected from Glu or Thr. In some embodiments, X_{31} is a basic or small amino acid residue, e.g., Xaa_{31} is selected from Arg or Thr. In some embodiments, Xaa_{32} is a small or neutral/polar amino acid residue, e.g., Xaa_{32} is selected from Ser or Asn. In some embodiments, Xaa_{33} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{33} is selected from Asn or Asp. In some embodiments, Xaa_{34} is a basic or small amino acid residue, e.g., Xaa_{34} is selected from Arg or Pro. In some embodiments, Xaa_{35} is an acidic or hydrophobic amino acid residue, e.g., Xaa_{35} is selected from Asp or Val. In some embodiments, Xaa_{36} is a basic or small amino acid residue, e.g., Xaa_{36} is selected from Arg or Ser. In some embodiments, Xaa_{37} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{37} is selected from Cys or Leu. In some embodiments, Xaa_{38} is a hydrophobic or small amino acid residue, e.g., Xaa_{38} is selected from Phe or Ala. In some embodiments, Xaa_{39} is a basic or hydrophobic amino acid residue, e.g., Xaa_{39} is selected from Lys or Val.

[0050] In some embodiments, Xaa_{40} is a basic or neutral/polar amino acid residue, e.g., Xaa_{40} is selected from Arg or Gln. In some embodiments, X_{41} is an acidic or small amino acid residue, e.g., Xaa_{41} is selected from Asp or Ser. In some embodiments, Xaa_{42} is a small or basic amino acid residue, e.g., Xaa_{42} is selected from Thr or Lys. In some embodiments, Xaa_{43} is a small or acidic amino acid residue, e.g., Xaa_{43} is selected from Ser or Asp. In some embodiments, Xaa_{44} is a hydrophobic or small amino acid residue, e.g., Xaa_{44} is selected from Ile or Thr. In some embodiments, Xaa_{45} is an acidic or small amino acid residue, e.g., Xaa_{45} is selected from Glu or Pro. In some embodiments, Xaa_{46} is a small or hydrophobic amino acid residue, e.g., Xaa_{46} is selected from Ser or Val. In some embodiments, Xaa_{47} is a small or acidic amino acid residue, e.g., Xaa_{47} is selected from Gly or Glu. In some embodiments, Xaa_{48} is a neutral/polar or small amino acid residue, e.g., Xaa_{48} is selected from

Gln or Thr. In some embodiments, Xaa_{49} is a small or acidic amino acid residue, e.g., Xaa_{49} is selected from Ala or Asp.

[0051] In some embodiments, Xaa_{50} is a hydrophobic or small amino acid residue, e.g., Xaa_{50} is selected from Tyr or Ser. In some embodiments, X_{51} is an acidic or small amino acid residue, e.g., Xaa_{51} is selected from Glu or Ser. In some embodiments, Xaa_{52} is a basic or small amino acid residue, e.g., Xaa_{52} is selected from Lys or Ser. In some embodiments, Xaa_{53} is a neutral/polar or basic amino acid residue, e.g., Xaa_{53} is selected from Cys or His. In some embodiments, Xaa_{54} is a small or basic amino acid residue, e.g., Xaa_{54} is selected from Gly or Arg. In some embodiments, Xaa_{55} is a small or acidic amino acid residue, e.g., Xaa_{55} is selected from Ser or Glu. In some embodiments, Xaa_{56} is a hydrophobic or small amino acid residue, e.g., Xaa_{56} is selected from Val or Ala. In some embodiments, Xaa_{57} is a neutral/polar or small amino acid residue, e.g., Xaa_{57} is selected from Asn or Ser. In some embodiments, Xaa_{58} is absent or is a neutral/polar amino acid residue, e.g., Cys. In some embodiments, Xaa_{59} is a neutral/polar or small amino acid residue, e.g., Xaa_{59} is selected from Gln or Ser.

[0052] In some embodiments, Xaa_{60} is an acidic or small amino acid residue, e.g., Xaa_{60} is selected from Glu or Pro. In some embodiments, X_{61} is a basic or neutral/polar amino acid residue, e.g., Xaa_{61} is selected from His or Asn. In some embodiments, Xaa_{62} is a hydrophobic or small amino acid residue, e.g., Xaa_{62} is selected from Leu or Pro. In some embodiments, Xaa_{63} is a hydrophobic or acidic amino acid residue, e.g., Xaa_{63} is selected from Val or Glu. In some embodiments, Xaa_{64} is a small or basic amino acid residue, e.g., Xaa_{64} is selected from Gly or Lys. In some embodiments, Xaa_{65} is a basic or small amino acid residue, e.g., Xaa_{65} is selected from Lys or Ser. In some embodiments, Xaa_{66} is a small or hydrophobic amino acid residue, e.g., Xaa_{66} is selected from Ser or Leu. In some embodiments, Xaa_{67} is a basic or small amino acid residue, e.g., Xaa_{67} is selected from Lys or Thr. In some embodiments, Xaa_{68} is a small or basic amino acid residue, e.g., Xaa_{68} is selected from Ser or Arg. In some embodiments, Xaa_{69} is a hydrophobic or small amino acid residue, e.g., Xaa_{69} is selected from Leu or Ser.

[0053] In some embodiments, Xaa_{70} is a hydrophobic or basic amino acid residue, e.g., Xaa_{70} is selected from Val or His. In some embodiments, X_{71} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{71} is selected from Gln or Glu. In some embodiments, Xaa_{72} is an acidic or small amino acid residue, e.g., Xaa_{72} is selected from Glu or Ser. In some embodiments, Xaa_{73} is a basic or small amino acid residue, e.g., Xaa_{73} is selected from His or Ser. In some embodiments, Xaa_{74} is a small or acidic amino acid residue, e.g., Xaa_{74} is selected from Thr or Glu. In some embodiments, Xaa_{75} is a hydrophobic or small amino acid residue, e.g., Xaa_{75} is selected from Val or Thr. In some embodiments, Xaa_{76} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{76} is selected from Gln or Glu. In some embodiments, Xaa_{77} is a neutral/polar or small amino acid residue, e.g., Xaa_{77} is selected from Gln or Pro. In some embodiments, Xaa_{78} is an acidic or small amino acid residue, e.g., Xaa_{78} is

selected from Glu or Ala. In some embodiments, Xaa_{79} is a neutral/polar or small amino acid residue, e.g., Xaa_{79} is selected from Asn or Ser.

[0054] In some embodiments, Xaa_{80} is a hydrophobic or small amino acid residue, e.g., Xaa_{80} is selected from Val or Ala. In some embodiments, X_{81} is a hydrophobic or basic amino acid residue, e.g., Xaa_{81} is selected from Trp or Arg. In some embodiments, Xaa_{82} is a hydrophobic or basic amino acid residue, e.g., Xaa_{82} is selected from Leu or Arg. In some embodiments, Xaa_{83} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{83} is selected from Gln or Asp. In some embodiments, Xaa_{84} is a basic or neutral/polar amino acid residue, e.g., Xaa_{84} is selected from His or Asn. In some embodiments, Xaa_{85} is a basic or neutral/polar amino acid residue, e.g., Xaa_{85} is selected from His or Gln. In some embodiments, Xaa_{86} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{86} is selected from Gln or Leu. In some embodiments, Xaa_{87} is a basic or small amino acid residue, e.g., Xaa_{87} is selected from His or Thr. In some embodiments, Xaa_{88} is an acidic or small amino acid residue, e.g., Xaa_{88} is selected from Asp or Gly. In some embodiments, Xaa_{89} is a hydrophobic or basic amino acid residue, e.g., Xaa_{89} is selected from Leu or Arg.

[0055] In some embodiments, Xaa_{90} is a hydrophobic or basic amino acid residue, e.g., Xaa_{90} is selected from Tyr or Arg. In some embodiments, Xaa_{91} is an acidic or small amino acid residue, e.g., Xaa_{91} is selected from Glu or Thr. In some embodiments, Xaa_{92} is a neutral/polar or basic amino acid residue, e.g., Xaa_{92} is selected from Gln or His. In some embodiments, Xaa_{93} is a small or hydrophobic amino acid residue, e.g., Xaa_{93} is selected from Thr or Met. In some embodiments, Xaa_{94} is an acidic or neutral/polar amino acid residue, e.g., Xaa_{94} is selected from Glu or Gln. In some embodiments, Xaa_{95} is a small or neutral/polar amino acid residue, e.g., Xaa_{95} is selected from Gly or Asn.

[0056] In some embodiments, the domain corresponding to residues 537-1476 of Figure 2 comprises a sequence according to Formula (III):

Leu-Xaa_1-Ωaa_1-Xaa_2-Φaa_1-Phe-Baa_1-Xaa_3-Leu-Xaa_4-Arg-Ile-Baa_2-Val-Leu-Xaa_5-Φaa_2-Xaa_6-Xaa_7-Cys-Xaa_8-Φaa_3-Baa_3-Xaa_9-Leu-Pro-Xaa_{10}-Xaa_{11}-Φaa_4-Gly-Xaa_{12}-Leu-Xaa_{13}-Xaa_{14}-Leu-Arg-Tyr-Leu-Xaa_{15}-Φaa_5-Ser-Xaa_{16}-Asn-Σaa_1-Xaa_{17}-Ile-Gln-Arg-Leu-Pro-Glu-Ser-Φaa_6-Xaa_{18}-Ωaa_2-Leu-Xaa_{19}-Xaa_{20}-Leu-Gln-Σaa_2-Leu-Xaa_{21}-Leu-Xaa_{22}-Gly-Cys-Xaa_{23}-Leu-Xaa_{24}-Xaa_{25}-Φaa_7-Pro-Xaa_{26}-Σaa_3-Met-Ser-Baa_4-Leu-Φaa_8-Xaa_{27}-Leu-Arg-Gln-Leu-Baa_5-Xaa_{28}-Xaa_{29}-Xaa_{30}-$Åaa_1$-Φaa_9-Ile-Σaa_4-Ωaa_3-Ile-Xaa_{31}-Ωaa_4-Val-Gly-Baa_6-Leu-Ile-Xaa_{32}-Leu-Gln-Glu-Leu-Xaa_{33}-Ala-Φaa_{10}-Xaa_{34}-Val-Xaa_{35}-Xaa_{36}-Baa_7-Xaa_{37}-Gly-Xaa_{38}-Xaa_{39}-Φaa_{11}-Ala-Glu-Leu-Ser-Σaa_5-Φaa_{12}-Xaa_{40}-Gln-Leu-Baa_8-Σaa_6-Xaa_{41}-Leu-Xaa_{42}-Ile-Xaa_{43}-Asn-Leu-Xaa_{44}-Asn-Val-Xaa_{45}-Xaa_{46}-Xaa_{47}-Ωaa_5-Glu-Σaa_7-Xaa_{48}-Lys-Ala-Baa_9-Leu-Ωaa_6-Ωaa_7-Lys-Gln-Xaa_{49}-Leu-Ωaa_8-Xaa_{50}-Leu-$Åaa_2$-Leu-Ωaa_9-Trp-Ala-Xaa_{51}-Gly-Xaa_{52}-Xaa_{53}-Xaa_{54}-Xaa_{55}-Xaa_{56}-Xaa_{57}-Xaa_{58}-Glu-Xaa_{59}-Xaa_{60}-Xaa_{61}-Xaa_{62}-Ωaa_{10}-Ωaa_{11}-Val-Leu-Xaa_{63}-Gly-Leu-Xaa_{64}-Pro-His-Xaa_{65}-Xaa_{66}-Leu-Baa_{10}-Xaa_{67}-Leu-Σaa_8-Ile-Baa_{11}-Xaa_{68}-Tyr-Σaa_9-Gly-Σaa_{10}-Σaa_{11}-Xaa_{69}-Pro-Ser-Trp-

Φaa_{13}-Xaa_{70}-Xaa_{71}-Xaa_{72}-Φaa_{14}-Leu-Pro-Asn-Φaa_{15}-Xaa_{73}-Thr-Φaa_{16}-Baa_{12}-Leu-Ωaa_{12}-Xaa_{74}-Cys-Σaa_{12}-Arg-Leu-Xaa_{75}-Xaa_{76}-Leu-Σaa_{13}-Xaa_{77}-Φaa_{17}-Gly-Gln-Leu-Xaa_{78}-Xaa_{79}-Leu-Baa_{13}-Xaa_{80}-Leu-His-Φaa_{18}-Ωaa_{13}-Xaa_{81}-Met-Σaa_{14}-Xaa_{82}-Val-Baa_{14}-Gln-Φaa_{19}-Xaa_{83}-Xaa_{84}-Xaa_{85}-Φaa_{20}-Xaa_{86}-Gly-Xaa_{87}-Σaa_{15}-Ωaa_{14}-Xaa_{88}-Xaa_{89}-Xaa_{90}-Phe-Pro-Xaa_{91}-Leu-Glu-Xaa_{92}-Leu-Xaa_{93}-Φaa_{21}-Ωaa_{15}-Ωaa_{16}-Met-Pro-Σaa_{16}-Leu-Ωaa_{17}-Glu-Φaa_{22} (III)

wherein: each of $\Phi_{1\text{-}22}$ is independently selected from any hydrophobic amino acid residue,
each of $\Sigma aa_{1\text{-}16}$ is independently selected from any small amino acid residue,
each of $Baa_{1\text{-}14}$ is independently selected from any basic amino acid residue,
each of $Åaa_{1\text{-}2}$ is independently selected from any acidic amino acid residue,
each of $\Omega aa_{1\text{-}16}$ is independently selected from any charged amino acid residue, and
$Xaa_{1\text{-}93}$ are each independently selected from any amino acid residue.

[0057] In some embodiments, Ωaa_1 is selected from His or Asp. In some embodiments, Ωaa_2 is selected from Asp or Arg. In some embodiments, Ωaa_3 is selected from Lys or Asp. In some embodiments, Ωaa_4 is selected from Glu or Lys. In some embodiments, Ωaa_5 is selected from Glu or Arg. In some embodiments, Ωaa_6 is selected from His or Asp. In some embodiments, Ωaa_7 is selected from Arg or Glu. In some embodiments, Ωaa_8 is selected from Glu or Lys. In some embodiments, Ωaa_9 is selected from Glu or Arg.

[0058] In some embodiments, Ωaa_{10} is selected from Glu or Arg. In some embodiments, Ωaa_{11} is selected from Glu or Lys. In some embodiments, Ωaa_{12} is selected from Lys or Asp. In some embodiments, Ωaa_{13} is selected from Lys or Glu. In some embodiments, Ωaa_{14} is selected from Lys or Glu. In some embodiments, Ωaa_{15} is selected from Glu or Arg. In some embodiments, Ωaa_{16} is selected from Asp or Arg.

[0059] In some embodiments, Φaa_1 is selected from Leu or Met. In some embodiments, Φaa_2 is selected from Leu or Phe. In some embodiments, Φaa_3 is selected from Met or Ile. In some embodiments, Φaa_4 is selected from Ile or Val. In some embodiments, Φaa_5 is selected from Ile or Leu. In some embodiments, Φaa_6 is selected from Leu or Val. In some embodiments, Φaa_7 is selected from Phe or Leu. In some embodiments, Φaa_8 is selected from Ile or Leu. In some embodiments, Φaa_9 is selected from Ile or Val.

[0060] In some embodiments, Φaa_{10} is selected from Phe or Tyr. In some embodiments, Φaa_{11} is selected from Leu or Ile. In some embodiments, Φaa_{12} is selected from Leu or Met. In some embodiments, Φaa_{13} is selected from Leu or Met. In some embodiments, Φaa_{14} is selected from Met or Tyr. In some embodiments, Φaa_{15} is selected from Leu or Met. In some embodiments, Φaa_{16} is selected from Leu or Ile. In some embodiments, Φaa_{17} is selected from Ile or Leu. In some

embodiments, Φaa_{18} is selected from Met or Ile. In some embodiments, Φaa_{19} is selected from Met or Ile.

[0061] In some embodiments, Φaa_{20} is selected from Leu or Phe. In some embodiments, Φaa_{21} is selected from Leu or Ile. In some embodiments, Φaa_{22} is selected from Phe or Trp.

[0062] In some embodiments, Baa_1, Baa_{3-6} and Baa_{9-14} are each independently selected from Arg or Lys. In some embodiments, Baa_2 and Baa_8 are each independently selected from His or Arg. In some embodiments, Baa_2 is selected from His or Lys.

[0063] In some embodiments, Σaa_1 is selected from Ala or Thr. In some embodiments, Σaa_2 is selected from Ala or Thr. In some embodiments, Σaa_3 is selected from Gly or Ser. In some embodiments, Σaa_4 is selected from Ser or Ala. In some embodiments, Σaa_5 is selected from Gly or Ala. In some embodiments, Σaa_6 is selected from Gly or Ser. In some embodiments, Σaa_7 is selected from Ala or Ser. In some embodiments, Σaa_8 is selected from Thr or Ser. In some embodiments, Σaa_9 is selected from Ser or Gly.

[0064] In some embodiments, Σaa_{10} is selected from Ala or Thr. In some embodiments, Σaa_{11} is selected from Thr or Ser. In some embodiments, Σaa_{12} is selected from Thr or Ala. In some embodiments, Σaa_{13} is selected from Ser or Pro. In some embodiments, Σaa_{14} is selected from Pro or Ser. In some embodiments, Σaa_{15} is selected from Thr or Gly. In some embodiments, Σaa_{16} is selected from Thr or Ser.

[0065] In some embodiments, each of $Åaa_{1-2}$ is independently selected from Asp or Glu.

[0066] In some embodiments Xaa_1 is a small or hydrophobic amino acid residue, e.g., Xaa_1 is selected from Pro or Leu. In some embodiments, Xaa_2 is an small or basic amino acid residue, e.g., Xaa_2 is selected from Ser or Arg. In some embodiments, Xaa_3 is a basic or hydrophobic amino acid residue, e.g., Xaa_3 is selected from Arg or Met. In some embodiments, Xaa_4 is an basic or small amino acid residue, e.g., Xaa_4 is selected from Lys or Ser. In some embodiments, Xaa_5 is a hydrophobic or acidic amino acid residue, e.g., Xaa_5 is selected from Val or Asp. In some embodiments, Xaa_6 is a neutral/polar or small amino acid residue, e.g., Xaa_6 is selected from Gln or Ser. In some embodiments, Xaa_7 is a basic or neutral/polar amino acid residue, e.g., Xaa_7 is selected from Lys or Asn. In some embodiments, Xaa_8 is a small or hydrophobic amino acid residue, e.g., Xaa_8 is selected from Gly or Val. In some embodiments, Xaa_9 is an acidic or neutral/polar amino acid residue, e.g., Xaa_9 is selected from Glu or Asn.

[0067] In some embodiments, Xaa_{10} is an acidic or small amino acid residue, e.g., Xaa_{10} is selected from Asp or Ser. In some embodiments, Xaa_{11} is a hydrophobic or small amino acid residue, e.g., Xaa_{11} is selected from Ile or Ser. In some embodiments, Xaa_{12} is an acidic or neutral/polar amino

acid residue, e.g., Xaa_{12} is selected from Asp or Asn. In some embodiments, Xaa_{13} is a hydrophobic or basic amino acid residue, e.g., Xaa_{13} is selected from Ile or Lys. In some embodiments, Xaa_{14} is a neutral/polar or basic amino acid residue, e.g., Xaa_{14} is selected from Gln or His. In some embodiments, Xaa_{15} is acidic or small amino acid residue, e.g., Xaa_{15} is selected from Asp or Gly. In some embodiments, Xaa_{16} is absent or is a hydrophobic amino acid residue, e.g., Tyr. In some embodiments, Xaa_{17} is a neutral/polar or basic amino acid residue, e.g., Xaa_{17} is selected from Cys or Arg. In some embodiments, Xaa_{18} is a neutral/polar or small amino acid residue, e.g., Xaa_{18} is selected from Cys or Thr. In some embodiments, Xaa_{19} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{19} is selected from Tyr or Cys.

[0068] In some embodiments, Xaa_{20} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{20} is selected from Asn or Leu. In some embodiments, Xaa_{21} is a basic or hydrophobic amino acid residue, e.g., Xaa_{21} is selected from Arg or Leu. In some embodiments, Xaa_{22} is a hydrophobic or acidic amino acid residue, e.g., Xaa_{22} is selected from Trp or Glu. In some embodiments, Xaa_{23} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{23} is selected from Gln or Glu. In some embodiments, Xaa_{24} is a basic or neutral/polar amino acid residue, e.g., Xaa_{24} is selected from Arg or Cys. In some embodiments, Xaa_{25} is a small or basic amino acid residue, e.g., Xaa_{25} is selected from Ser or Arg. In some embodiments, Xaa_{26} is a neutral/polar or basic amino acid residue, e.g., Xaa_{26} is selected from Gln or Arg. In some embodiments, Xaa_{27} is a neutral/polar or basic amino acid residue, e.g., Xaa_{27} is selected from Asn or Lys. In some embodiments, Xaa_{28} is a hydrophobic or small amino acid residue, e.g., Xaa_{28} is selected from Val or Ala. In some embodiments, Xaa_{29} is an acidic or neutral/polar amino acid residue, e.g., Xaa_{29} is selected from Glu or Asn.

[0069] In some embodiments, Xaa_{30} is an acidic or small amino acid residue, e.g., Xaa_{30} is selected from Asp or Pro. In some embodiments, X_{31} is a hydrophobic or small amino acid residue, e.g., Xaa_{31} is selected from Tyr or Ala. In some embodiments, Xaa_{32} is a small or acidic amino acid residue, e.g., Xaa_{32} is selected from Ser or Glu. In some embodiments, Xaa_{33} is a small or basic amino acid residue, e.g., Xaa_{33} is selected from Ser or Lys. In some embodiments, Xaa_{34} is a basic or neutral/polar amino acid residue, e.g., Xaa_{34} is selected from Lys or Asn. In some embodiments, Xaa_{35} is an hydrophobic or acidic amino acid residue, e.g., Xaa_{35} is selected from Leu or Asp. In some embodiments, Xaa_{36} is a neutral/polar or basic amino acid residue, e.g., Xaa_{36} is selected from Asn or His. In some embodiments, Xaa_{37} is a neutral/polar or basic amino acid residue, e.g., Xaa_{37} is selected from Asn or Lys. In some embodiments, Xaa_{38} is a neutral/polar or basic amino acid residue, e.g., Xaa_{38} is selected from Asn or Lys. In some embodiments, Xaa_{39} is a basic or small amino acid residue, e.g., Xaa_{39} is selected from Lys or Gly.

[0070] In some embodiments, Xaa_{40} is a small or neutral/polar amino acid residue, e.g., Xaa_{40} is selected from Thr or Asn. In some embodiments, X_{41} is an small or acidic amino acid residue, e.g., Xaa_{41} is selected from Thr or Asp. In some embodiments, Xaa_{42} is a basic or small amino acid residue,

e.g., Xaa_{42} is selected from Arg or Ser. In some embodiments, Xaa_{43} is a small or basic amino acid residue, e.g., Xaa_{43} is selected from Thr or Arg. In some embodiments, Xaa_{44} is an acidic or neutral/polar amino acid residue, e.g., Xaa_{44} is selected from Glu or Gln. In some embodiments, Xaa_{45} is a small or acidic amino acid residue, e.g., Xaa_{45} is selected from Gly or Glu. In some embodiments, Xaa_{46} is a small or basic amino acid residue, e.g., Xaa_{46} is selected from Ser or Lys. In some embodiments, Xaa_{47} is a basic or small amino acid residue, e.g., Xaa_{47} is selected from Lys or Thr. In some embodiments, Xaa_{48} is a small or basic amino acid residue, e.g., Xaa_{48} is selected from Ser or Arg. In some embodiments, Xaa_{49} is a hydrophobic or basic amino acid residue, e.g., Xaa_{49} is selected from Tyr or Lys.

[0071] In some embodiments, Xaa_{50} is a small or hydrophobic amino acid residue, e.g., Xaa_{50} is selected from Ala or Leu. In some embodiments, X_{51} is a hydrophobic or acidic amino acid residue, e.g., Xaa_{51} is selected from Ala or Asp. In some embodiments, Xaa_{52} is a neutral/polar or basic amino acid residue, e.g., Xaa_{52} is selected from Gln or Arg. In some embodiments, Xaa_{53} is a hydrophobic or small amino acid residue, e.g., Xaa_{53} is selected from Val or Gly. In some embodiments, Xaa_{54} is absent or is a small amino acid residue, e.g., Ser. In some embodiments, Xaa_{55} is absent or is a small amino acid residue, e.g., Ser. In some embodiments, Xaa_{56} is absent or is a hydrophobic amino acid residue, e.g., Leu. In some embodiments, Xaa_{57} is an acidic or hydrophobic amino acid residue, e.g., Xaa_{57} is selected from Glu or Ala. In some embodiments, Xaa_{58} is a basic or small amino acid residue, e.g., Xaa_{58} is selected from His or Gly. In some embodiments, Xaa_{59} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{59} is selected from Leu or Cys.

[0072] In some embodiments, Xaa_{60} is a hydrophobic or acidic amino acid residue, e.g., Xaa_{60} is selected from Leu or Asp. In some embodiments, X_{61} is a hydrophobic or basic amino acid residue, e.g., Xaa_{61} is selected from Val or Arg. In some embodiments, Xaa_{62} is a small or acidic amino acid residue, e.g., Xaa_{62} is selected from Ser or Asp. In some embodiments, Xaa_{63} is a hydrophobic or basic amino acid residue, e.g., Xaa_{63} is selected from Leu or Lys. In some embodiments, Xaa_{64} is a neutral/polar or basic amino acid residue, e.g., Xaa_{64} is selected from Gln or Arg. In some embodiments, Xaa_{65} is a basic or small amino acid residue, e.g., Xaa_{65} is selected from His or Pro. In some embodiments, Xaa_{66} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{66} is selected from Phe or Asn. In some embodiments, Xaa_{67} is a small or acidic amino acid residue, e.g., Xaa_{67} is selected from Ser or Glu. In some embodiments, Xaa_{68} is a small or hydrophobic amino acid residue, e.g., Xaa_{68} is selected from Gly or Tyr. In some embodiments, Xaa_{69} is a hydrophobic or small amino acid residue, e.g., Xaa_{69} is selected from Val or Ser.

[0073] In some embodiments, Xaa_{70} is an acidic or small amino acid residue, e.g., Xaa_{70} is selected from Asp or Thr. In some embodiments, X_{71} is a hydrophobic or acidic amino acid residue, e.g., Xaa_{71} is selected from Val or Asp. In some embodiments, Xaa_{72} is a basic or neutral/polar amino acid residue, e.g., Xaa_{72} is selected from Lys or Gln. In some embodiments, Xaa_{73} is a small or acidic

amino acid residue, e.g., Xaa_{73} is selected from Gly or Glu. In some embodiments, Xaa_{74} is a neutral/polar or small amino acid residue, e.g., Xaa_{74} is selected from Asn or Ser. In some embodiments, Xaa_{75} is an acidic or small amino acid residue, e.g., Xaa_{75} is selected from Glu or Thr. In some embodiments, Xaa_{76} is a small or acidic amino acid residue, e.g., Xaa_{76} is selected from Gly or Glu. In some embodiments, Xaa_{77} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{77} is selected from Tyr or Cys. In some embodiments, Xaa_{78} is a hydrophobic or basic amino acid residue, e.g., Xaa_{78} is selected from Phe or His. In some embodiments, Xaa_{79} is a basic or hydrophobic amino acid residue, e.g., Xaa_{79} is selected from His or Ile.

[0074] In some embodiments, Xaa_{80} is a hydrophobic or basic amino acid residue, e.g., Xaa_{80} is selected from Val or His. In some embodiments, X_{81} is a small or hydrophobic amino acid residue, e.g., Xaa_{81} is selected from Arg or Gly. In some embodiments, Xaa_{82} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{82} is selected from Val or Gln. In some embodiments, Xaa_{83} is a small or neutral/polar amino acid residue, e.g., Xaa_{83} is selected from Ser or Asn. In some embodiments, Xaa_{84} is a basic or hydrophobic amino acid residue, e.g., Xaa_{84} is selected from His or Leu. In some embodiments, Xaa_{85} is a neutral/polar or acidic amino acid residue, e.g., Xaa_{85} is selected from Gln or Glu. In some embodiments, Xaa_{86} is a neutral/polar or hydrophobic amino acid residue, e.g., Xaa_{86} is selected from Cys or Tyr. In some embodiments, Xaa_{87} is a neutral/polar or small amino acid residue, e.g., Xaa_{87} is selected from Cys or Thr. In some embodiments, Xaa_{88} is a small or hydrophobic amino acid residue, e.g., Xaa_{88} is selected from Ser or Val. In some embodiments, Xaa_{89} is a basic or small amino acid residue, e.g., Xaa_{89} is selected from Lys or Ser.

[0075] In some embodiments, Xaa_{90} is a hydrophobic or small amino acid residue, e.g., Xaa_{90} is selected from Leu or Gly. In some embodiments, Xaa_{91} is a basic or hydrophobic amino acid residue, e.g., Xaa_{91} is selected from Arg or Leu. In some embodiments, Xaa_{92} is and acidic or hydrophobic amino acid residue, e.g., Xaa_{92} is selected from Glu or Leu. In some embodiments, Xaa_{93} is a hydrophobic or neutral/polar amino acid residue, e.g., Xaa_{93} is selected from Val or Asn.

[0076] In yet another aspect, the invention provides isolated polynucleotides comprising a nucleotide sequence encoding at least one domain as broadly described above.

[0077] In still another aspect, the invention provides antigen-binding molecules that are specifically immuno-interactive with a polypeptide or portion as broadly described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078] Figure 1 is a schematic representation of the location and structure of the eight main classes of plant disease resistance proteins.

[0079] Figure 2 is a diagrammatic representation showing an alignment of the amino acid sequences set forth in SEQ ID NO: 2 and 4 using ClustalW multiple alignment and the PAM250 similarity matrix as disclosed for example by Dayhoff *et al.* (1978) A model of evolutionary change in proteins. Matrices for determining distance relationships *In* M. O. Dayhoff, (ed.), Atlas of protein sequence and structure, Vol. 5, pp. 345-358, National Biomedical Research Foundation, Washington DC; and by Gonnet *et al.*, 1992, *Science* **256**(5062): 144301445.

[0080] Figure 3 is a photographic representation showing the migration of amplification products on an agarose gel following an RT-PCR using primers specific for each banana NBS class using template RNA from *M. acuminata ssp. malaccensis* resistant (R) or susceptible (S) plants. Total RNA was extracted from leaf or root tissue and treated with DNAase. C+ lanes, expected ~ 480 bp actin 1 cDNA fragment; C- lanes, no reverse transcriptase; AD, expected ~580 bp actin 1 genomic DNA fragment with ~100 bp intron included.

TABLE A

BRIEF DESCRIPTION OF THE SEQUENCES

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 1	Nucleotide sequence of RGA5 obtained from Calcutta 4	4380 nts
SEQ ID NO: 2	Deduced amino acid sequence encoded by SEQ ID NO: 1	1441 aa
SEQ ID NO: 3	Nucleotide sequence of RGA2 obtained from *Musa acuminata* spp *malaccensis*	3699 nts
SEQ ID NO: 4	Deduced amino acid sequence encoded by SEQ ID NO: 1	1232 aa

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0081] Unless stated otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. The following terms are defined below. These definitions are for illustrative purposes and are not intended to limit the common meaning in the art of the defined terms.

[0082] The articles "*a*" and "*an*" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0083] By "*about*" is meant a quantity, level, value, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, frequency, percentage, dimension, size, amount, weight or length.

[0084] By "*antigen-binding molecule*" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

[0085] As used herein, the term "*binds specifically,*" "*specifically immuno-interactive*" and the like refers to antigen-binding molecules that bind or a immuno-interactive with the polypeptide or polypeptide portions of the invention but do not significantly bind to homologous prior art polypeptides.

[0086] By "*biologically active portion*" is meant a portion of a full-length parent peptide or polypeptide which portion retains an activity of the parent molecule. For example, a biologically active portion of polypeptide of the invention will retain the ability to confer disease resistance, especially resistance to fungal pathogens such as *Fusarium*. As used herein, the term "*biologically active portion*" includes deletion mutants and peptides, for example of at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous amino acids, which comprise an activity of a parent molecule. Portions of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesised using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "*Peptide Synthesis*" by Atherton and Shephard which is included in a publication entitled "*Synthetic Vaccines*" edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a peptide or polypeptide of the

invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques. Recombinant nucleic acid techniques can also be used to produce such portions.

[0087] As used herein, the term "*cis-acting sequence*," "*cis-acting element*" or "*cis-regulatory region*" or "*regulatory region*" or similar term shall be taken to mean any sequence of nucleotides, which when positioned appropriately relative to an expressible genetic sequence, is capable of regulating, at least in part, the expression of the genetic sequence. Those skilled in the art will be aware that a *cis*-regulatory region may be capable of activating, silencing, enhancing, repressing or otherwise altering the level of expression and/or cell-type-specificity and/or developmental specificity of a gene sequence at the transcriptional or post-transcriptional level. In certain embodiments of the present invention, the *cis*-acting sequence is an activator sequence that enhances or stimulates the expression of an expressible genetic sequence.

[0088] Throughout this specification, unless the context requires otherwise, the words "*comprise*," "*comprises*" and "*comprising*" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

[0089] By "*corresponds to*" or "*corresponding to*" is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

[0090] As used herein, the terms "*culturing*", "*culture*" and the like refer to the set of procedures used *in vitro* where a population of cells (or a single cell) is incubated under conditions which have been shown to support the growth or maintenance of the cells *in vitro*. The art recognises a wide number of formats, media, temperature ranges, gas concentrations etc. which need to be defined in a culture system. The parameters will vary based on the format selected and the specific needs of the individual who practices the methods herein disclosed. However, it is recognised that the determination of culture parameters is routine in nature.

[0091] By "*disease resistance*" is intended that plants avoid or suppress the disease symptoms that are the outcome of plant-pathogen interaction. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms. The methods of the invention can be utilised to protect plants from disease, particularly those diseases that are caused by plant pathogens, such as Fusarium wilt.

[0092] By "*expression vector*" is meant any autonomous genetic element capable of directing the transcription of a polynucleotide contained within the vector and suitably the synthesis of a peptide or polypeptide encoded by the polynucleotide. Such expression vectors are known to practitioners in the art.

[0093] The term "*gene*" as used herein refers to any and all discrete coding regions of the cell's genome, as well as associated non-coding and regulatory regions. The gene is also intended to mean the open reading frame encoding specific polypeptides, introns, and adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression. In this regard, the gene may further comprise control signals such as promoters, enhancers, termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control signals. The DNA sequences may be cDNA or genomic DNA or a fragment thereof. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host.

[0094] The terms "*growing*" or "*regeneration*" as used herein mean growing a whole, differentiated plant from a plant cell, a group of plant cells, a plant part (including seeds), or a plant piece (e.g., from a protoplast, callus, or tissue part).

[0095] "*Hybridisation*" is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA U pairs with A and C pairs with G. In this regard, the terms "match" and "mismatch" as used herein refer to the hybridisation potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridise efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridise efficiently.

[0096] Reference herein to "*immuno-interactive*" includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0097] By "*isolated*" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide", as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally-occurring state, e.g., a DNA fragment which has been removed from the sequences that are normally adjacent to the fragment. Alternatively, an "isolated peptide" or an "isolated polypeptide" and the like, as used herein, refer to *in vitro* isolation and/or purification of a peptide or polypeptide molecule from its natural cellular environment, and from association with other components of the cell, i.e., it is not associated with *in vivo* substances.

[0098] By "*marker gene*" is meant a gene that imparts a distinct phenotype to cells expressing the marker gene and thus allows such transformed cells to be distinguished from cells that do not have

the marker. A selectable marker gene confers a trait for which one can 'select' based on resistance to a selective agent (e.g., a herbicide, antibiotic, radiation, heat, or other treatment damaging to untransformed cells). A screenable marker gene (or reporter gene) confers a trait that one can identify through observation or testing, *i.e.*, by 'screening' (e.g., β-glucuronidase, luciferase, or other enzyme activity not present in untransformed cells).

[0099] As used herein, a "*naturally-occurring*" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature. For example a naturally-occurring nucleic acid molecule can encode a natural protein.

[0100] By "*obtained from*" is meant that a sample such as, for example, a nucleic acid extract or polypeptide extract is isolated from, or derived from, a particular source. For example, the extract may be isolated directly from plants, especially monocotyledonous plants and more especially non-graminaceous monocotyledonous plants such as banana.

[0101] The term "*oligonucleotide*" as used herein refers to a polymer composed of a multiplicity of nucleotide residues (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof, including nucleotides with modified or substituted sugar groups and the like) linked *via* phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotide residues and linkages between them are naturally-occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphorothioate, phosphorodithioate, phophoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule can vary depending on the particular application. Oligonucleotides are a polynucleotide subset with 200 bases or fewer in length. Preferably, oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g., for probes; although oligonucleotides may be double stranded, e.g., for use in the construction of a variant nucleic acid sequence. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0102] The term "*operably connected*" or "*operably linked*" as used herein means placing a structural gene under the regulatory control of a promoter, which then controls the transcription and optionally translation of the gene. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting; i.e. the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence

element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting; i.e., the genes from which it is derived.

[0103] The term "*pathogen*" is used herein in its broadest sense to refer to an organism or an infectious agent whose infection of cells of viable plant tissue elicits a disease response.

[0104] The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0105] The terms "*polynucleotide variant*" and "*variant*" refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridise with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that vary from a reference polynucleotide by addition, deletion or substitution of at least one nucleotide. In this regard, it is well understood in the art, for example, that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains a biological function or activity of the reference polynucleotide. The terms "*polynucleotide variant*" and "*variant*" also include naturally-occurring allelic variants.

[0106] "*Polypeptide*," "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0107] The term "*polypeptide variant*" refers to polypeptides which are distinguished from a reference polypeptide by the addition, deletion or substitution of at least one amino acid residue. In certain embodiments, one or more amino acid residues of a reference polypeptide are replaced by different amino acids. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide (conservative substitutions) as described hereinafter.

[0108] By "*primer*" is meant an oligonucleotide which, when paired with a strand of DNA, is capable of initiating the synthesis of a primer extension product in the presence of a suitable polymerising agent. The primer is preferably single-stranded for maximum efficiency in amplification but can alternatively be double-stranded. A primer must be sufficiently long to prime the synthesis of extension products in the presence of the polymerisation agent. The length of the primer depends on many factors, including application, temperature to be employed, template reaction conditions, other reagents, and source of primers. For example, depending on the complexity of the target sequence, the

oligonucleotide primer typically contains 15 to 35 or more nucleotide residues, although it can contain fewer nucleotide residues. Primers can be large polynucleotides, such as from about 35 nucleotides to several kilobases or more. Primers can be selected to be "substantially complementary" to the sequence on the template to which it is designed to hybridise and serve as a site for the initiation of synthesis. By "substantially complementary", it is meant that the primer is sufficiently complementary to hybridise with a target polynucleotide. Desirably, the primer contains no mismatches with the template to which it is designed to hybridise but this is not essential. For example, non-complementary nucleotide residues can be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotide residues or a stretch of non-complementary nucleotide residues can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridise therewith and thereby form a template for synthesis of the extension product of the primer.

[0109] "*Probe*" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically refers to a polynucleotide probe that binds to another polynucleotide, often called the "target polynucleotide", through complementary base pairing. Probes can bind target polynucleotides lacking complete sequence complementarity with the probe, depending on the stringency of the hybridisation conditions. Probes can be labelled directly or indirectly.

[0110] The term "*recombinant polynucleotide*" as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

[0111] By "*recombinant polypeptide*" is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant or synthetic polynucleotide.

[0112] By "*regulatory element*" or "*regulatory element*" is meant nucleic acid sequences (e.g., DNA) necessary for expression of an operably linked coding sequence in a particular host cell. The regulatory sequences that are suitable for prokaryotic cells for example, include a promoter, and optionally a *cis*-acting sequence such as an operator sequence and a ribosome binding site. Control sequences that are suitable for eukaryotic cells include promoters, polyadenylation signals, transcriptional enhancers, translational enhancers, leader or trailing sequences that modulate mRNA stability, as well as targeting sequences that target a product encoded by a transcribed polynucleotide to an intracellular compartment within a cell or to the extracellular environment.

[0113] The term "*sequence identity*" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of

comparison. Thus, a "*percentage of sequence identity*" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "*sequence identity*" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

[0114] "*Similarity*" refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in Table B *infra*. Similarity may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* 12, 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0115] Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity" and "substantial identity". A "*reference sequence*" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e., only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "*comparison window*" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference

also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, *Nucl. Acids Res.* **25**:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0116] By the term *"taxon"* herein is meant a unit of botanical classification. It thus includes, genus, species, cultivars, varieties, variants and other minor taxonomic groups which lack a consistent nomenclature.

[0117] The term "*transformation*" means alteration of the genotype of an organism, for example a bacterium, yeast or plant, by the introduction of a foreign or endogenous nucleic acid.

[0118] By "*vector*" is meant a polynucleotide molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, yeast or virus, into which a polynucleotide can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector can also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are known to those of skill in the art.

2. Modulation of disease resistance

[0119] The invention is drawn to polynucleotides, polypeptides and methods for modulating disease resistance, especially for stimulating or enhancing disease resistance in plants, caused by pathogens. Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, insects, nematodes, fungi, and the like. Viruses include any plant virus, for example, tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf mosaic virus, etc. Specific fungal and viral pathogens for the major crops include:

[0120] Soybeans: *Phytophthora megasperma* fsp. *glycinea*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae*

(*Phomopsis sojae*), *Diaporthe phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora sojina*, *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotichum truncatum*), *Corynespora cassiicola*, *Septoria glycines*, *Phyllosticta sojicola*, *Alternaria alternata*, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*, *Phialophora gregata*, Soybean mosaic virus, *Glomerella glycines*, Tobacco Ring spot virus, Tobacco Streak virus, *Phakopsorapachyrhizi*, *Pythium aphanidermatum*, *Pythium ultimum*, *Pythium debaryanum*, Tomato spotted wilt virus, *Heterodera glycines Fusarium solani*;

[0121] Canola: *Albugo candida*, *Alternaria brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Mycosphaerella brassiccola*, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium roseum*, *Alternaria alternata*;

[0122] Alfalfa: *Clavibater michiganese* subsp. *insidiosum*, *Pythium ultimum*, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*, *Pythium aphanidermatum*, *Phytophthora megasperma*, *Peronospora trifoliorum*, *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Leptotrochila medicaginis*, *Fusarium*, *Xanthomonas campestris* p.v. *alfalfae*, *Aphanomyces euteiches*, *Stemphylium herbarum*, *Stemphylium alfalfae*;

[0123] Wheat: *Pseudomonas syringae* p.v. *atrofaciens*, *Urocystis agropyri*, *Xanthomonas campestris* p.v. *translucens*, *Pseudomonas syringae* p.v. *syringae*, *Alternaria alternata*, *Cladosporium herbarum*, *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Ustilago tritici*, *Ascochyta tritici*, *Cephalosporium gramineum*, *Collotetrichum graminicola*, *Erysiphe graminis* f.sp. *tritici*, *Puccinia graminis* f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, *Puccinia striiformis*, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*, *Septoria avenae*, *Pseudocercosporella herpotrichoides*, *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris sorokiniana*, Barley Yellow Dwarf Virus, Brome Mosaic Virus, Soil Borne Wheat Mosaic Virus, Wheat Streak Mosaic Virus, Wheat Spindle Streak Virus, American Wheat Striate Virus, *Claviceps purpurea*, *Tilletia tritici*, *Tilletia laevis*, *Ustilago tritici*, *Tilletia indica*, *Rhizoctonia solani*, *Pythium arrhenomannes*, *Pythium gramicola*, *Pythium aphanidermatum*, High Plains Virus, European wheat striate virus;

[0124] Sunflower: *Plasmophora halstedii*, *Sclerotinia sclerotiorum*, *Aster Yellows*, *Septoria helianthi*, *Phomopsis helianthi*, *Alternaria helianthi*, *Alternaria zinniae*, *Botrytis cinerea*, *Phoma macdonaldii*, *Macrophomina phaseolina*, *Erysiphe cichoracearum*, *Rhizopus oryzae*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, *Puccinia helianthi*, *Verticillium dahliae*, *Erwinia carotovorum* pv. *carotovora*, *Cephalosporium acremonium*, *Phytophthora cryptogea*, *Albugo tragopogonis*;

[0125] Corn: *Fusarium moniliforme* var. *subglutinans*, *Erwinia stewartii*, *Fusarium moniliforme*, *Gibberella zeae* (*Fusarium graminearum*), *Stenocarpella maydi* (*Diplodia maydis*), *Pythium*

irregulare, *Pythium debaryanum*, *Pythium graminicola*, *Pythium splendens*, *Pythium ultimum*, *Pythium aphanidermatum*, *Aspergillus flavus*, *Bipolaris maydis* O, T (*Cochliobolus heterostrophus*), *Helminthosporium carbonum* I, II & III (*Cochliobolus carbonum*), *Exserohilum turcicum* I, II & III, *Helminthosporium pedicellatum*, *Physoderma maydis*, *Phyllosticta maydis*, *Kabatiella-maydis*, *Cercospora sorghi*, *Ustilago maydis*, *Puccinia sorghi*, *Puccinia polysora*, *Macrophomina phaseolina*, *Penicillium oxalicum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia pallescens*, *Clavibacter michiganense* subsp. *nebraskense*, *Trichoderma viride*, Maize Dwarf Mosaic Virus A & B, Wheat Streak Mosaic Virus, Maize Chlorotic Dwarf Virus, *Claviceps sorghi*, *Pseudonomas avenae*, *Erwinia chrysanthemi* pv. *zea*, *Erwinia carotovora*, Corn stunt spiroplasma, *Diplodia macrospora*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora maydis*, *Peronosclerospora sacchari*, *Sphacelotheca reiliana*, *Physopella zeae*, *Cephalosporium maydis*, *Cephalosporium acremonium*, Maize Chlorotic Mottle Virus, High Plains Virus, Maize Mosaic Virus, Maize Rayado Fino Virus, Maize Streak Virus, Maize Stripe Virus, Maize Rough Dwarf Virus;

[0126] Sorghum: *Exserohilum turcicum*, *Colletotrichum graminicola* (*Glomerella graminicola*), *Cercospora sorghi*, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*, *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*, *Fusarium moniliforme*, *Alternaria alternata*, *Bipolaris sorghicola*, *Helminthosporium sorghicola*, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas avenae* (*Pseudomonas alboprecipitans*), *Ramulispora sorghi*, *Ramulispora sorghicola*, *Phyllachara sacchari*, *Sporisorium reilianum* (*Sphacelotheca reiliana*), *Sphacelotheca cruenta*, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthona macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*, etc.

[0127] Nematodes include parasitic nematodes such as root-knot, cyst, lesion, and reniform nematodes, etc

[0128] Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Coleoptera and Lepidoptera. Insect pests of the invention for the major crops include:

[0129] Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, surgarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis* barberi,

northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus* spp., wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplusfemurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscrurus*, grass thrips; *Solenopsis milesta*, thief ant; Tetranychus urticae, twospotted spider mite;

[0130] Sorghum: *Chilo partellus*, sorghum borer; *Spodopterafrugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes*, *Conoderus*, and *Aeolus* spp., wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*; corn leaf aphid; Sipha flava, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata* howardi, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplusfemurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; Thrips tabaci, onion thrips; *Franklinkiella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite;

[0131] Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea, grape colaspis*; *Lissorhoptrus oryzophilus*, rice

water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug;

[0132] Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatalis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestani*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite;

[0133] Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* ssp., Root maggots.

[0134] In certain embodiments, the plant pathogen is selected from fungi, especially soil borne fungi such as *Fusarium oxysporum*, *Verticillium dahliae*, *Cladosporium* and *Ralstona Solanaceum*.

3. Polynucleotides of the invention

[0135] The present invention is predicated, in part, on the isolation of two novel R genes from banana, one from *Musa acuminata* Calcutta 4 and the other from *Musa acuminata* spp *malaccensis*. The Calcutta 4 gene designated RGA5 is 4380 nts long and comprises a single open reading frame of 4321 nts that encodes a 1441-aa putative polypeptide product. The nucleotide sequence of this gene and its deduced polypeptide sequence are presented in SEQ ID NO: 1 and 2, respectively. The *Musa acuminata* spp *malaccensis* gene designated RGA2 comprises a single open reading frame of 3699 nts, which encodes a putative polypeptide product of 1232 aa. The nucleotide sequence of the RGA2 gene and its deduced polypeptide sequence are presented in SEQ ID NO: 3 and 4, respectively.

[0136] In accordance with the present invention, the novel R genes will be useful for facilitating the construction of crop plants that are resistant to pathogenic disease, especially disease caused by fungal pathogens, viruses, nematodes, insects and the like. The R genes of the present invention can also be used as markers in genetic mapping as well as in assessing disease resistance in a plant of interest. Thus, the sequences can be used in breeding programs. See, for example, Gentzbittel *et al.* (1998, *Theor. Appl. Genet.* 96:519-523). Additional uses for the sequences of the invention include using the sequences as bait to isolate other signalling components on defence/resistance pathways and to isolate the corresponding promoter sequences. The sequences may also be used to modulate plant

development processes, such as pollen development, regulation of organ shape, differentiation of aleurone and shoot epidermis, embryogenic competence, and cell/cell interactions. See, generally, Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.). The sequences of the present invention can also be used to generate variants (e.g., by 'domain swapping') for the generation of new resistance specificities.

[0137] The invention encompasses isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the nucleic acid molecule or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or polypeptide is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesised. Suitably, an "isolated" polynucleotide is free of sequences (especially protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide was derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide was derived. A polypeptide that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, culture medium suitably represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

[0138] The present invention also encompasses portions of the disclosed nucleotide sequences. Portions of a nucleotide sequence may encode polypeptide portions or segments that retain the biological activity of the native polypeptide and hence modulate or regulate disease resistance. Alternatively, portions of a nucleotide sequence that are useful as hybridisation probes generally do not encode amino acid sequences retaining such biological activity. Thus, portions of a nucleotide sequence may range from at least about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 80, 90, 100 nucleotides, or almost up to the full-length nucleotide sequence encoding the polypeptides of the invention.

[0139] A portion of an R nucleotide sequence that encodes a biologically active portion of an R polypeptide of the invention will encode at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous amino acid residues, or almost up to the total number of amino acids present in a full-length R polypeptide of the invention (for example, 1440 or 1330 amino acid residues for SEQ ID

NO: 2 or 4, respectively). Portions of an R nucleotide sequence that are useful as hybridisation probes or PCR primers generally need not encode a biologically active portion of an R polypeptide.

[0140] Thus, a portion of an R nucleotide sequence may encode a biologically active portion of an R polypeptide, or it may be a fragment that can be used as a hybridisation probe or PCR primer using standard methods known in the art. A biologically active portion of an R polypeptide can be prepared by isolating a portion of one of the R nucleotide sequences of the invention, expressing the encoded portion of the R polypeptide (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the R polypeptide. Nucleic acid molecules that are portions of an R nucleotide sequence comprise at least about 15, 16, 17, 18, 19, 20, 25, 30, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or 650 nucleotides, or almost up to the number of nucleotides present in a full-length R nucleotide sequence disclosed herein (for example, 4375 or 3690 nucleotides for SEQ ID NO: 1 or 14, respectively).

[0141] The invention also contemplates variants of the disclosed nucleotide sequences. Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologues (different locus), and orthologues (different organism) or can be non naturally occurring. Naturally occurring variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridisation techniques as known in the art. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product). For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the R polypeptides of the invention. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode an R polypeptide of the invention. Generally, variants of a particular nucleotide sequence of the invention will have at least about 30%, 40% 50%, 55%, 60%, 65%, 70%, generally at least about 75%, 80%, 85%, desirably about 90% to 95% or more, and more suitably about 98% or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

[0142] The nucleotide sequences of the invention can be used to isolate corresponding sequences from other organisms, particularly other plants. Methods are readily available in the art for the hybridisation of nucleic acid sequences. Coding sequences from other plants may be isolated according to well known techniques based on their sequence identity with the coding sequences set forth herein. In these techniques all or part of the known coding sequence is used as a probe which selectively hybridises to other R coding sequences present in a population of cloned genomic DNA

fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism. Accordingly, the present invention also contemplates polynucleotides that hybridise to the R gene nucleotide sequences, or to their complements, under stringency conditions described below. As used herein, the term "hybridises under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridisation and washing. Guidance for performing hybridisation reactions can be found in Ausubel *et al.*, (1998, *supra*), Sections 6.3.1-6.3.6. Aqueous and non-aqueous methods are described in that reference and either can be used. Reference herein to low stringency conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridisation at 42° C, and at least about 1 M to at least about 2 M salt for washing at 42° C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 2× SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at room temperature. One embodiment of low stringency conditions includes hybridisation in 6× sodium chloride/sodium citrate (SSC) at about 45° C, followed by two washes in 0.2× SSC, 0.1% SDS at least at 50° C (the temperature of the washes can be increased to 55° C for low stringency conditions). Medium stringency conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridisation at 42° C, and at least about 0.1 M to at least about 0.2 M salt for washing at 55° C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 2× SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at 60-65° C. One embodiment of medium stringency conditions includes hybridising in 6× SSC at about 45° C, followed by one or more washes in 0.2× SSC, 0.1% SDS at 60° C. High stringency conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridisation at 42° C, and about 0.01 M to about 0.02 M salt for washing at 55° C. High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 0.2× SSC, 0.1% SDS; or (ii) 0.5% BSA, 1mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 1% SDS for washing at a temperature in excess of 65° C. One embodiment of high stringency conditions includes hybridising in 6× SSC at about 45° C, followed by one or more washes in 0.2× SSC, 0.1% SDS at 65° C.

[0143] In certain embodiments, an isolated nucleic acid molecule of the invention hybridises under very high stringency conditions. One embodiment of very high stringency conditions includes hybridising 0.5M sodium phosphate, 7% SDS at 65° C, followed by one or more washes at 0.2× SSC, 1% SDS at 65° C.

[0144] Other stringency conditions are well known in the art and a skilled addressee will recognise that various factors can be manipulated to optimise the specificity of the hybridisation. Optimisation of the stringency of the final washes can serve to ensure a high degree of hybridisation. For detailed examples, see Ausubel *et al.*, *supra* at pages 2.10.1 to 2.10.16 and Sambrook *et al.* (1989, *supra*) at sections 1.101 to 1.104.

[0145] While stringent washes are typically carried out at temperatures from about 42° C to 68° C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridisation rate typically occurs at about 20° C to 25° C below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T_m are well known in the art (see Ausubel *et al.*, *supra* at page 2.10.8). In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula:

$$T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600/\text{length})$$

wherein: M is the concentration of Na^+, preferably in the range of 0.01 molar to 0.4 molar; %G+C is the sum of guanosine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex. The T_m of a duplex DNA decreases by approximately 1° C with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at T_m – 15° C for high stringency, or T_m – 30° C for moderate stringency.

[0146] In one example of a hybridisation procedure, a membrane (e.g., a nitrocellulose membrane or a nylon membrane) containing immobilised DNA is hybridised overnight at 42° C in a hybridisation buffer (50% deionised formamide, 5× SSC, 5x Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrollidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing labelled probe. The membrane is then subjected to two sequential medium stringency washes (i.e., 2× SSC, 0.1% SDS for 15 min at 45° C, followed by 2× SSC, 0.1% SDS for 15 min at 50° C), followed by two sequential higher stringency washes (i.e., 0.2× SSC, 0.1% SDS for 12 min at 55° C followed by 0.2× SSC and 0.1%SDS solution for 12 min at 65-68° C.

[0147] Variant nucleotide sequences also encompass sequences derived from a mutagenic or recombinogenic procedures such as 'DNA shuffling' which can be used for swapping domains in a polypeptide of interest with domains of other polypeptides. With DNA shuffling, one or more different R coding sequences can be manipulated to create a new R sequence possessing desired properties. In this procedure, libraries of recombinant polynucleotides are generated from a population of related polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest (e.g., the coiled coil domain, the NBS domain and/or the LRR domain

of the polypeptides of the invention) may be shuffled between the R gene of the invention and other known R genes to obtain a new gene coding for a protein with an improved property of interest, such broadening spectrum of disease resistance. Illustrative resistance R genes that could be employed for this purpose are listed in Table B below.

5

TABLE B

PLANT DISEASE RESISTANCE GENES CLONED FROM 1994 TO 2003.

PLANT-PATHOGEN INTERACTION		PLANT (R) PROTEIN STRUCTURE	R PROTEIN NAME	REFERENCE
Flax	*Melampsora lini*	TIR-NBS-LRR	L	Lawrence *et al.*, 1995
Tobacco	Tobacco mosaic virus	TIR-NBS-LRR	N	Whitman *et al.*, 1996
Flax	*Melampsora lini*	TIR-NBS-LRR	M	Anderson *et al.*, 1997
Arabidopsis	*Peronospora parasitica*	TIR-NBS-LRR	RPP 5	Parker *et al.*, 1997
Arabidopsis	*Pseudomonas*	TIR-NBS-LRR	RPS4	Gassmann *et al.*, 1999
Flax	*Melampsora lini*	TIR-NBS-LRR	P	Dodds *et al.*, 2000
Arbidopsis	*Pseudomonas syringae*	NBS-LRR	RPS2	Mindrinos *et al.*, 1994
Tomato	*Pseudomonas syringae*	NBS-LRR	Prf	Salmeron *et al.*, 1996
Arbidopsis	*Pseudomonas syringae*	NBS-LRR	RPM1	Grant *et al.*, 1996
Wheat	*Heterodera avenae*	NBS-LRR	Cre3	Lagudah *et al.*, 1997
Tomato	Fusarium oxysporum	NBS-LRR	12	Simons *et al.*, 1998
Tomato	Meloidogyne sp	NBS-LRR	Mi	Milligan *et al.*, 1998
Tomato	*Macrosiphum euphorbie*	NBS-LRR	Mi	Milligan *et al.*, 1998
Arabidopsis	*Peronospora parasitica*	NBS-LRR	RPP1	Botella *et al.*, 1998
Lettuce	*Bremia lactucae*	NBS-LRR	Dm3	Meyers *et al.*, 1998
Rice	*Xanthomonas*	NBS-LRR	Xa1	Yoshimura *et al.*, 1998
Arabidopsis	*Pseudomonas*	NBS-LRR	RPS5	Warren *et al.*, 1998
Maize	*Puccinia sorghi*	NBS-LRR	Rp1-D	Collins *et al.*, 1999
Pepper	*Xanthomonas campestris*	NBS-LRR	Bs2	Thai *et al.*, 1999
Potato	PVX	NBS-LRR	Rx2	Bendahmane *et al.*, 1999
Rice	*Magnaporthe*	NBS-LRR	Pi-ta	Bryan *et al.*, 2000
Barley	*Blumeria graminis*	NBS-LRR	Mla	Zhou *et al.*, 2000
Arabidopsis	*Peronospora parasitica*	NBS-LRR	RPP 13	Bittner-Eddy *et al.*, 2000

PLANT-PATHOGEN INTERACTION		PLANT (R) PROTEIN STRUCTURE	R PROTEIN NAME	REFERENCE
Tomato	Tospovirus	NBS-LRR	Sw-5	Brommonschenkel *et al.*.2000
Potato	*Globodera pallida*	NBS-LRR	Gpa 2	Van der Vossen *et al.*, 2000
Potato	*Phytophtora infestans*	NBS-LRR	R1	Ballvora *et al.*, 2002
Tomato	*Globodera rostochiensis*	NBS-LRR	Hero	Ernst *et al.*, 2002
Potato	*Phytophthora infestans*	NBS-LRR	RB	Song et al., 2003

[0148] Strategies for DNA shuffling are known in the art. See, for example: Stemmer (1994, *Proc. Natl. Acad. Sci. USA* **91**:10747-10751; 1994, *Nature* **370**:389-391); Crameri *et al.* (1997, *Nature Biotech.* **15**:436-438); Moore *et al.* (1997, *J. Mol. Biol.* **272**:336-347); Zhang *et al.* (1997 *Proc. Natl. Acad. Sci. USA* **94**:4504-4509); Crameri *et al.* (1998, *Nature* **391**:288-291); and U.S. Pat. Nos. 5,605,793 and 5,837,458.

4. Polypeptides of the invention

[0149] The present invention provides polypeptides and biologically active portions thereof that confer resistance to disease, especially resistance to pathogenic disease including disease caused by fungal pathogen, viruses, nematodes, insects and the like. Biologically active portions of the R polypeptides of the invention include portions with immuno-interactive activity of at least about 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60 amino acid residues in length. For example, immuno-interactive fragments contemplated by the present invention are at least 6 and desirably at least 8 amino acid residues in length, which can elicit an immune response in an animal for the production of antigen-binding molecules that are immuno-interactive with the R polypeptides of the invention. Such antigen-binding molecules can be used to screen organisms, especially plants, for structurally and/or functionally related R polypeptides. Typically, portions of the disclosed R polypeptides may participate in an interaction, e.g., an intramolecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction (e.g., the interaction can be transient and a covalent bond is formed or broken). An inter-molecular interaction can be between an R polypeptide and a pathogen elicitor protein. Biologically active portions of an R polypeptide include peptides comprising amino acid sequences sufficiently similar to or derived from the amino acid sequences of the disclosed R polypeptides, e.g., the amino acid sequences shown in SEQ ID NO: 2 or 4, which include less amino acids than the full-length R polypeptide, and exhibit at least one activity of an R polypeptide. Typically, biologically active portions comprise a domain or motif with at least one activity of the R polypeptide, e.g., the ability to bind to a pathogen elicitor protein or to confer disease resistance. A biologically active portion of an R polypeptide can be a polypeptide which is, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,

24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 300, 400, 500, 600, 700, 800, 900 or 1000 or more amino acids in length. Suitably, the portion is a "biologically-active portion" having no less than about 1%, 10%, 25% 50% of the pathogen elicitor protein-binding activity or the resistance-conferring activity of the full-length polypeptide.

[0150] The present invention also contemplates variant R polypeptides. "Variant" polypeptides include proteins derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, modulating disease resistance or interacting with a pathogen elicitor protein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native R protein of the invention will have at least 40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, preferably about 90% to 95% or more, and more preferably about 98% or more sequence similarity with the amino acid sequence for the native protein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0151] The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the R proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985, *Proc. Natl. Acad. Sci. USA* **82**:488-492), Kunkel *et al.* (1987, *Methods in Enzymol.* **154**:367-382), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.* ("Molecular Biology of the Gene", Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al. (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.). Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property are known in the art. Such methods are adaptable for rapid screening of the gene libraries generated by combinatorial mutagenesis of R polypeptides. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify R polypeptide variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6:327-331). Conservative

substitutions, such as exchanging one amino acid with another having similar properties, may be desirable as discussed in more detail below.

[0152] Variant polypeptides may contain conservative amino acid substitutions at various locations along their sequence, as compared to the R polypeptide amino acid sequences of the invention. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, which can be generally sub-classified as follows:

[0153] Acidic: The residue has a negative charge due to loss of H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH. Amino acids having an acidic side chain include glutamic acid and aspartic acid.

[0154] Basic: The residue has a positive charge due to association with H ion at physiological pH or within one or two pH units thereof (e.g., histidine) and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH. Amino acids having a basic side chain include arginine, lysine and histidine.

[0155] Charged: The residues are charged at physiological pH and, therefore, include amino acids having acidic or basic side chains (i.e., glutamic acid, aspartic acid, arginine, lysine and histidine).

[0156] Hydrophobic: The residues are not charged at physiological pH and the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. Amino acids having a hydrophobic side chain include tyrosine, valine, isoleucine, leucine, methionine, phenylalanine and tryptophan.

[0157] Neutral/polar: The residues are not charged at physiological pH, but the residue is not sufficiently repelled by aqueous solutions so that it would seek inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. Amino acids having a neutral/polar side chain include asparagine, glutamine, cysteine, histidine, serine and threonine.

[0158] This description also characterises certain amino acids as "small" since their side chains are not sufficiently large, even if polar groups are lacking, to confer hydrophobicity. With the exception of proline, "small" amino acids are those with four carbons or less when at least one polar group is on the side chain and three carbons or less when not. Amino acids having a small side chain include glycine, serine, alanine and threonine. The gene-encoded secondary amino acid proline is a special case due to its known effects on the secondary conformation of peptide chains. The structure of proline differs from all the other naturally-occurring amino acids in that its side chain is bonded to the

nitrogen of the α-amino group, as well as the α-carbon. Several amino acid similarity matrices (e.g., PAM120 matrix and PAM250 matrix as disclosed for example by Dayhoff *et al.* (1978) A model of evolutionary change in proteins. Matrices for determining distance relationships *In* M. O. Dayhoff, (ed.), Atlas of protein sequence and structure, Vol. 5, pp. 345-358, National Biomedical Research Foundation, Washington DC; and by Gonnet *et al.*, 1992, *Science* **256**(5062): 144301445), however, include proline in the same group as glycine, serine, alanine and threonine. Accordingly, for the purposes of the present invention, proline is classified as a "small" amino acid.

[0159] The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the invention have been classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behaviour.

[0160] Amino acid residues can be further sub-classified as cyclic or noncyclic, and aromatic or nonaromatic, self-explanatory classifications with respect to the side-chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of four carbon atoms or less, inclusive of the carboxyl carbon, provided an additional polar substituent is present; three or less if not. Small residues are, of course, always nonaromatic. Dependent on their structural properties, amino acid residues may fall in two or more classes. For the naturally-occurring protein amino acids, sub-classification according to the this scheme is presented in the Table C.

TABLE C

AMINO ACID SUB-CLASSIFICATION

SUB-CLASSES	AMINO ACIDS
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine
Residues that influence chain orientation	Glycine and Proline

[0161] Accordingly, the present invention also contemplates variants of the naturally occurring or parent R polypeptide sequences or their biologically-active fragments, wherein the variants are

distinguished from the parent sequences by the addition, deletion, or substitution of one or more amino acids. In general, variants display at least about 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 % similarity to a parent R polypeptide sequence as for example set forth in SEQ ID NO: 2 or 4. Desirably, variants will have at least 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 % sequence identity to a parent R polypeptide sequence as set forth in SEQ ID NO:2 or 4. Moreover, sequences differing from the native or parent sequences by the addition, deletion, or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60 ,70, 80 ,90, 100, 150, 200, 300, 500 or more amino acids but which retain the disease-resistance-conferring or pathogen elicitor-binding properties are contemplated. Polypeptides of the invention include polypeptides that are encoded by polynucleotides that hybridise under stringency conditions as defined herein, especially high stringency conditions, to the polynucleotide sequences of the invention, or the non-coding strand thereof, as described above.

[0162] In one embodiment, variant polypeptides differ from the disclosed sequences by at least one but by less than 50, 40, 30, 20, 15, 10, 8, 6, 5, 4, 3, 2 or 1 amino acid residue(s). In another, variant polypeptides differ from the corresponding sequence in SEQ ID NO: 2 or 4 by at least 1% but less than 20%, 15%, 10% or 5% of the residues. (If this comparison requires alignment the sequences should be aligned for maximum similarity. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, suitably, differences or changes at a non-essential residue or a conservative substitution.

[0163] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of an R polypeptide without abolishing or substantially altering one or more of its activities (e.g., disease-resistance or pathogen elicitor-binding properties). Suitably, the alteration does not substantially alter one of these activities, e.g., the activity is at least 20%, 40%, 60%, 70% or 80% of wild-type. An "essential" amino acid residue is a residue that, when altered from the wild-type sequence of an R polypeptide of the invention, results in abolition of disease-resistance or pathogen elicitor-binding properties such that less than 20% of the wild-type activity is present. For example, conserved amino acid residues between the R polypeptides shown in Figure 2 may be unamenable to alteration.

[0164] Desirable variant R polypeptides are those having conserved amino acid substitutions. Examples of conservative substitutions include the following: aspartic-glutamic as acidic amino acids; lysine/arginine/histidine as basic amino acids; serine/glycine/alanine/threonine as small amino acids; leucine/isoleucine, methionine/valine, alanine/valine as hydrophobic amino acids. Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side

chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional R polypeptide can readily be determined by assaying its disease resistance-conferring activity or its pathogen-elicitor-binding activity. Conservative substitutions are shown in Table D below under the heading of exemplary substitutions. More preferred substitutions are shown under the heading of preferred substitutions. Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. After the substitutions are introduced, the variants are screened for biological activity.

TABLE D

EXEMPLARY AND PREFERRED AMINO ACID SUBSTITUTIONS

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS	PREFERRED SUBSTITUTIONS
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleu	Leu
Leu	Norleu, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS	PREFERRED SUBSTITUTIONS
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala, Norleu	Leu

[0165] Alternatively, similar amino acids for making conservative substitutions can be grouped into three categories based on the identity of the side chains. The first group includes glutamic acid, aspartic acid, arginine, lysine, histidine, which all have charged side chains; the second group includes glycine, serine, threonine, cysteine, tyrosine, glutamine, asparagine; and the third group includes leucine, isoleucine, valine, alanine, proline, phenylalanine, tryptophan, methionine, as described in Zubay, G., *Biochemistry*, third edition, Wm.C. Brown Publishers (1993).

[0166] Thus, a predicted non-essential amino acid residue in an R polypeptide is typically replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of an R gene coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for disease resistance-conferring activity or pathogen-elicitor-binding activity to identify mutants that retain activity. Following mutagenesis of the coding sequences, the encoded peptide can be expressed recombinantly and the activity of the peptide can be determined.

[0167] In other embodiments, variant R polypeptides include an amino acid sequence having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98% or more similarity to a corresponding sequence of SEQ ID NO: 2 or 4, and has disease resistance-conferring activity or pathogen-elicitor-binding activity.

[0168] The R polypeptides of the present invention contain a significant number of structural characteristics in common with each other as for example depicted in Figure 2. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally-occurring and can be from either the same or different species. Members of a family can also have common functional characteristics.

5. Anti-R polypeptide antigen-binding molecules

[0169] The invention also provides an antigen-binding molecule that is specifically immuno-interactive with an R polypeptide of the invention. In one embodiment, the antigen-binding molecule comprise whole polyclonal antibodies. Such antibodies may be prepared, for example, by injecting a polypeptide, portion or variant of the invention into a production species, which may include mice or rabbits, to obtain polyclonal antisera. Methods of producing polyclonal antibodies are well known to

those skilled in the art. Exemplary protocols which may be used are described for example in Coligan *et al.*, CURRENT PROTOCOLS IN IMMUNOLOGY, (John Wiley & Sons, Inc, 1991), and Ausubel *et al.*, (1994-1998, *supra*), in particular Section III of Chapter 11.

[0170] In lieu of polyclonal antisera obtained in a production species, monoclonal antibodies may be produced using the standard method as described, for example, by Köhler and Milstein (1975, *Nature* **256**, 495-497), or by more recent modifications thereof as described, for example, in Coligan *et al.*, (1991, *supra*) by immortalising spleen or other antibody producing cells derived from a production species which has been inoculated with one or more of the polypeptides, fragments, variants or derivatives of the invention.

[0171] The invention also contemplates as antigen-binding molecules Fv, Fab, Fab' and $F(ab')_2$ immunoglobulin fragments. Alternatively, the antigen-binding molecule may comprise a synthetic stabilised Fv fragment. Exemplary fragments of this type include single chain Fv fragments (sFv, frequently termed scFv) in which a peptide linker is used to bridge the N terminus or C terminus of a V_H domain with the C terminus or N-terminus, respectively, of a V_L domain. ScFv lack all constant parts of whole antibodies and are not able to activate complement. ScFvs may be prepared, for example, in accordance with methods outlined in Kreber *et al* (Kreber *et al.* 1997, *J. Immunol. Methods*; **201(1)**: 35-55). Alternatively, they may be prepared by methods described in U.S. Patent No 5,091,513, European Patent No 239,400 or the articles by Winter and Milstein (1991, *Nature* **349**:293) and Plückthun *et al* (1996, In *Antibody engineering: A practical approach*. 203-252). In another embodiment, the synthetic stabilised Fv fragment comprises a disulphide stabilised Fv (dsFv) in which cysteine residues are introduced into the V_H and V_L domains such that in the fully folded Fv molecule the two residues will form a disulphide bond therebetween. Suitable methods of producing dsFv are described for example in (Glockscuther *et al. Biochem.* **29**: 1363-1367; Reiter *et al.* 1994, *J. Biol. Chem.* **269**: 18327-18331; Reiter *et al.* 1994, *Biochem. 33*: 5451-5459; Reiter *et al.* 1994. *Cancer Res.* **54**: 2714-2718; Webber *et al.* 1995, *Mol. Immunol.* **32**: 249-258).

[0172] Phage display and combinatorial methods for generating R polypeptide antigen-binding molecules are known in the art (as described in, e.g., Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-

4137; and Barbas et al. (1991) *PNAS* 88:7978-7982). The antigen-binding molecules can be used to screen expression libraries for variant R polypeptides. They can also be used to detect and/or isolate the R polypeptides of the invention. Thus, the invention also contemplates the use of antigen-binding molecules to isolate R polypeptides using , for example, any suitable immunoaffinity based method including, but not limited to, immunochromatography and immunoprecipitation. A suitable method utilises solid phase adsorption in which anti-R polypeptide antigen-binding molecules are attached to a suitable resin, the resin is contacted with a sample suspected of containing a R polypeptide, and the R polypeptide, if any, is subsequently eluted from the resin. Illustrative resins include: Sepharose® (Pharmacia), Poros® resins (Roche Molecular Biochemicals, Indianapolis), Actigel Superflow™ resins (Sterogene Bioseparations Inc., Carlsbad Calif.), and Dynabeads™ (Dynal Inc., Lake Success, N.Y.).

[0173] The antigen-binding molecule can be coupled to a compound, e.g., a label such as a radioactive nucleus, or imaging agent, e.g., a radioactive, enzymatic, or other, e.g., imaging agent, e.g., a NMR contrast agent. Labels which produce detectable radioactive emissions or fluorescence are preferred. An anti-R polypeptide antigen-binding molecule (e.g., monoclonal antibody) can be used to detect R polypeptides (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-R polypeptides antigen-binding molecules can be used diagnostically to monitor R polypeptides levels in tissue as part of an agronomic testing procedure. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H. The label may be selected from a group including a chromogen, a catalyst, an enzyme, a fluorophore, a chemiluminescent molecule, a lanthanide ion such as Europium (Eu^{34}), a radioisotope and a direct visual label. In the case of a direct visual label, use may be made of a colloidal metallic or non-metallic particle, a dye particle, an enzyme or a substrate, an organic polymer, a latex particle, a liposome, or other vesicle containing a signal producing substance and the like.

[0174] A large number of enzymes useful as labels is disclosed in United States Patent Specifications U.S. 4,366,241, U.S. 4,843,000, and U.S. 4,849,338. Enzyme labels useful in the present invention include alkaline phosphatase, horseradish peroxidase, luciferase, β-galactosidase,

glucose oxidase, lysozyme, malate dehydrogenase and the like. The enzyme label may be used alone or in combination with a second enzyme in solution.

6. Nucleic acid constructs

6.1 Prokaryotic expression

[0175] The present invention further relates to a nucleic acid construct designed for genetic transformation of prokaryotic cells, comprising a polynucleotide, portion or variant according to the invention operably linked to a regulatory sequence, which will typically comprise a transcriptional control element or promoter. Suitably, the chimeric construct is operable in a Gram-negative prokaryotic cell. A variety of prokaryotic expression vectors, which may be used as a basis for constructing the chimeric nucleic acid construct, may be utilised to express a polynucleotide, portion or variant according to the invention. These include but are not limited to a chromosomal vector (e.g., a bacteriophage such as bacteriophage λ), an extrachromosomal vector (e.g., a plasmid or a cosmid expression vector). The expression vector will also typically contain an origin of replication, which allows autonomous replication of the vector, and one or more genes that allow phenotypic selection of the transformed cells. Any of a number of suitable promoter sequences, including constitutive and inducible promoter sequences, may be used in the expression vector (see e.g., Bitter, *et al.*, 1987, *Methods in Enzymology* **153**: 516-544). For example, inducible promoters such as pL of bacteriophage γ, plac, ptrp, ptac ptrp-lac hybrid promoter and the like may be used. The nucleic acid construct may then be used to transform the desired prokaryotic host cell to produce a recombinant prokaryotic host cell, e.g., for producing a recombinant R polypeptide.

6.2 Eukaryotic expression

[0176] The invention also contemplates a nucleic acid construct designed for expressing a polynucleotide, portion or variant of the invention in a eukaryotic host cell. A variety of eukaryotic host-expression vector systems may be utilised in this regard. These include, but are not limited to, yeast transformed with recombinant yeast expression vectors; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus); or animal cell systems infected with recombinant virus expression vectors (e.g., retroviruses, adenovirus, Vaccinia virus), or transformed animal cell systems engineered for stable expression. In certain advantageous embodiments, the chimeric nucleic acid construct is designed for genetic transformation of plants as described hereinafter.

6.3 Plant expression

[0177] In accordance with the present invention, it is proposed that the R gene polynucleotides, portions and variants of the invention will be useful for facilitating the construction of crop plants that are resistant to pathogenic disease, including diseases caused by fungal pathogens, viruses, nematodes,

insects and the like. Accordingly, the present invention also relates to operably linking a polynucleotide, portion or variant of as described herein to a regulatory sequence (e.g., a promoter and a 3' non-translated region) that is function in plants to create a nucleic acid construct, designed for genetic transformation of plants.

6.3.1 Plant promoters

[0178] Numerous promoters that are active in plant cells have been described in the literature, illustrative examples of which include the nopaline synthase (NOS) promoter, the octopine synthase (OCS) promoter (which is carried on tumour-inducing plasmids of Agrobacterium tumefaciens), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter and the CaMV 35S promoter, the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter, the sucrose synthase promoter, the R gene complex promoter, the GST-II-27 gene promoter and the chlorophyll a/b binding protein gene promoter, etc.

[0179] For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is desirable that the promoters driving expression of the target gene have relatively high expression in these specific tissues. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea, the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat, the nuclear photosynthetic ST-LS1 promoter from potato, the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from Arabidopsis thaliana. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (Larix laricina), the promoter for the cab gene, cab6, from pine, the promoter for the Cab-1 gene from wheat, the promoter for the CAB-1 gene from spinach, the promoter for the cab1R gene from rice, the pyruvate, orthophosphate dikinase (PPDK) promoter from corn, the promoter for the tobacco Lhcb1*2 gene, the Arabidopsis thaliana SUC2 sucrose-H+ symporter and the promoter for the thylakoid membrane proteins from spinach (psaD, psaF, psaE, PC, FNR, atpC, atpD, cab, rbcS). Other promoters for the chlorophyll a/b-binding proteins may also be utilised in the invention, such as the promoters for LhcB gene and PsbP gene from white mustard.

[0180] For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of corn, wheat, rice and barley, it is desirable that the promoters driving expression of the target gene have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or tuber-enhanced expression are known, including the class I patatin promoter, the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter, the promoter for the major tuber proteins including the 22 kd

protein complexes and protease inhibitors, the promoter for the granule-bound starch synthase gene (GBSS) and other class I and II patatins promoters.

[0181] Other promoters can also be used to express a target gene in specific tissues, such as seeds or fruits. Examples of such promoters include the 5' regulatory regions from such genes as napin, phaseolin, soybean trypsin inhibitor, ACP, stearoyl-ACP desaturase, soybean α' subunit of β-conglycinin (soy 7s), and oleosin. Further examples include the promoter for β-conglycinin. Also included are the zeins, which are a group of storage proteins found in corn endosperm. Genomic clones for zein genes have been isolated and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and genes, could also be used. Other promoters known to function, for example, in corn include the promoters for the following genes: waxy, Brittle, Shrunken 2, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. Examples of promoters suitable for expression in wheat include those promoters for the ADPglucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. Examples of such promoters for barley include those for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins.

[0182] Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene. Expression in root tissue could also be accomplished using the root specific subdomains of the CaMV35S promoter that have been identified.

[0183] Desirable promoters for expression in cultured cells are strong constitutive promoters, or promoters that respond to a specific inducer (Gatz and Lenk, 1998, *Trends Plant Science* 3: 352-8). In certain embodiments, nucleic acid constructs expressing R polynucleotides of the present invention are introduced into banana plants that are susceptible Exemplary constitutive promoters for expression in intact banana plants are described in International Publication No. WO 02/053744 and in co-pending PCT Application No. PCT/AU03/00919.

6.3.2 3' Non-translated region

[0184] The constructs of the present invention can comprise a 3' non-translated sequence. A 3' non-translated sequence refers to that portion of a gene comprising a DNA segment that contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is characterised by effecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognised by identity with the canonical form 5' AATAAA-3' although variations are not uncommon.

[0185] The 3' non-translated regulatory DNA sequence preferably includes from about 50 to 1,000 nucleotide base pairs and may contain plant transcriptional and translational termination sequences in addition to a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. Examples of suitable 3' non-translated sequences are the 3' transcribed non-translated regions containing a polyadenylation signal from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens* (Bevan *et al.*, 1983, *Nucl. Acid Res.*, **11**:369) and the terminator for the T7 transcript from the octopine synthase gene of *Agrobacterium tumefaciens*. Alternatively, suitable 3' non-translated sequences may be derived from plant genes such as the 3' end of the protease inhibitor I or II genes from potato or tomato, the soybean storage protein genes and the pea E9 small subunit of the ribulose-1,5-bisphosphate carboxylase (ssRUBISCO) gene, although other 3' elements known to those of skill in the art can also be employed. Alternatively, 3' non-translated regulatory sequences can be obtained *de novo* as, for example, described by An (1987, *Methods in Enzymology*, **153**:292).

6.3.3 Optional sequences

[0186] The nucleic acid construct of the present invention can further include enhancers, either translation or transcription enhancers, as may be required. These enhancer regions are well known to persons skilled in the art, and can include the ATG initiation codon and adjacent sequences. The initiation codon must be in phase with the reading frame of the coding sequence relating to the foreign or endogenous DNA sequence to ensure translation of the entire sequence. The translation control signals and initiation codons can be of a variety of origins, both natural and synthetic. Translational initiation regions may be provided from the source of the transcriptional initiation region, or from the foreign or endogenous DNA sequence. The sequence can also be derived from the source of the promoter selected to drive transcription, and can be specifically modified so as to increase translation of the mRNA.

[0187] Examples of transcriptional enhancers include, but are not restricted to, elements from the CaMV 35S promoter and octopine synthase genes as for example described by Last *et al.* (U.S. Patent No. 5,290,924). It is proposed that the use of an enhancer element such as the *ocs* element, and particularly multiple copies of the element, will act to increase the level of transcription from adjacent promoters when applied in the context of plant transformation. Alternatively, the omega sequence derived from the coat protein gene of the tobacco mosaic virus (Gallie *et al.*, 1987, *Nucleic Acids Res.* **15**(8):3257-73) may be used to enhance translation of the mRNA transcribed from a polynucleotide according to the invention.

[0188] As the DNA sequence inserted between the transcription initiation site and the start of the coding sequence, i.e., the untranslated leader sequence, can influence gene expression, one can also employ a particular leader sequence. Preferred leader sequences include those that comprise sequences

selected to direct optimum expression of the R polypeptide gene. For example, such leader sequences include a preferred consensus sequence which can increase or maintain mRNA stability and prevent inappropriate initiation of translation as for example described by Joshi (1987, *Nucl. Acid Res.*, 15:6643). However, other leader sequences, e.g., the leader sequence of RTBV, have a high degree of secondary structure that is expected to decrease mRNA stability and/or decrease translation of the mRNA. Thus, leader sequences (i) that do not have a high degree of secondary structure, (ii) that have a high degree of secondary structure where the secondary structure does not inhibit mRNA stability and/or decrease translation, or (iii) that are derived from genes that are highly expressed in plants, will be most preferred.

[0189] Regulatory elements such as the sucrose synthase intron as, for example, described by Vasil *et al.* (1989, *Plant Physiol.*, **91**:5175), the Adh intron I as, for example, described by Callis *et al.* (1987, *Genes Develop.*, II), or the TMV omega element as, for example, described by Gallie *et al.* (1989, *The Plant Cell*, 1:301) can also be included where desired. Other such regulatory elements useful in the practice of the invention are known to those of skill in the art.

[0190] Additionally, targeting sequences may be employed to target R polypeptide to an intracellular compartment within plant cells or to the extracellular environment. For example, a DNA sequence encoding a transit or signal peptide sequence may be operably linked to a sequence encoding the R polypeptide or biologically active portion thereof such that, when translated, the transit or signal peptide can transport the polypeptide or portion to a particular intracellular or extracellular destination, and can then be post-translationally removed. Transit or signal peptides act by facilitating the transport of proteins through intracellular membranes, e.g., endoplasmic reticulum, vacuole, vesicle, plastid, mitochondrial and plasmalemma membranes. For example, the targeting sequence can direct a desired protein to a particular organelle such as a vacuole or a plastid (e.g., a chloroplast), rather than to the cytosol. Thus, the nucleic acid construct can further comprise a plastid transit peptide encoding DNA sequence operably linked between a promoter region or promoter variant according to the invention and the foreign or endogenous DNA sequence. For example, reference may be made to Heijne *et al.* (1989, *Eur. J. Biochem.*, **180**:535) and Keegstra *et al.* (1989, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40**:471).

[0191] The nucleic acid construct is typically introduced into a vector, such as a plasmid. Plasmid vectors include additional DNA sequences that provide for easy selection, amplification, and transformation of the expression cassette in prokaryotic and eukaryotic cells, e.g., pUC-derived vectors, pSK-derived vectors, pGEM-derived vectors, pSP-derived vectors, or pBS-derived vectors. Additional DNA sequences include origins of replication to provide for autonomous replication of the vector, selectable marker genes, preferably encoding antibiotic or herbicide resistance, unique multiple cloning sites providing for multiple sites to insert DNA sequences or genes encoded in the nucleic acid construct, and sequences that enhance transformation of prokaryotic and eukaryotic cells.

[0192] The vector desirably contains an element(s) that permits either stable integration of the vector into the host cell genome or autonomous replication of the vector in the cell independent of the genome of the cell. The vector may be integrated into the host cell genome when introduced into a host cell. For integration, the vector may rely on a foreign or endogenous DNA sequence present therein or any other element of the vector for stable integration of the vector into the genome by homologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location in the chromosome. To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleic acids, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleic acid sequences.

[0193] For cloning and subcloning purposes, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in a host cell such as a bacterial cell. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAMβ1 permitting replication in *Bacillus*. The origin of replication may be one having a mutation to make its function temperature-sensitive in a *Bacillus* cell (see, e.g., Ehrlich, 1978, *Proc. Natl. Acad. Sci. USA* **75**:1433).

6.3.4 Marker genes

[0194] To facilitate identification of transformants, the nucleic acid construct desirably comprises a selectable or screenable marker gene as, or in addition to, a polynucleotide sequence according to the invention. The actual choice of a marker is not crucial as long as it is functional (i.e., selective) in combination with the plant cells of choice. The marker gene and the R polynucleotide sequence of interest do not have to be linked, since co-transformation of unlinked genes as, for example, described in U.S. Pat. No. 4,399,216 is also an efficient process in plant transformation.

[0195] Included within the terms selectable or screenable marker genes are genes that encode a "secretable marker" whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or secretable enzymes that can be detected by their catalytic activity. Secretable proteins include, but are not restricted to, proteins that are inserted or trapped in the cell wall (e.g., proteins that include a leader sequence such as that found in the expression unit of extensin or tobacco

PR-S); small, diffusible proteins detectable, e.g., by ELISA; and small active enzymes detectable in extracellular solution (e.g., α-amylase, β-lactamase, phosphinothricin acetyltransferase).

6.3.5 Selectable markers

[0196] Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers that confer antibiotic resistance such as ampicillin, kanamycin, erythromycin, chloramphenicol or tetracycline resistance. Exemplary selectable markers for selection of plant transformants include, but are not limited to, a *hyg* gene which encodes hygromycin B resistance; a neomycin phosphotransferase (*neo*) gene conferring resistance to kanamycin, paromomycin, G418 and the like as, for example, described by Potrykus *et al.* (1985, *Mol. Gen. Genet.* **199**:183); a glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides as, for example, described in EP-A 256 223; a glutamine synthetase gene conferring, upon overexpression, resistance to glutamine synthetase inhibitors such as phosphinothricin as, for example, described WO87/05327, an acetyl transferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin as, for example, described in EP-A 275 957, a gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine as, for example, described by Hinchee *et al.* (1988, *Biotech.*, **6**:915), a *bar* gene conferring resistance against bialaphos as, for example, described in WO91/02071; a nitrilase gene such as *bxn* from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker *et al.*, 1988, *Science*, **242**:419); a dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate (Thillet *et al.*, 1988, *J. Biol. Chem.*, **263**:12500); a mutant acetolactate synthase gene (ALS), which confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (EP-A-154 204); a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; or a dalapon dehalogenase gene that confers resistance to the herbicide.

6.3.6 Screenable markers

[0197] Preferred screenable markers include, but are not limited to, a *uidA* gene encoding a β-glucuronidase (GUS) enzyme for which various chromogenic substrates are known; a β-galactosidase gene encoding an enzyme for which chromogenic substrates are known; an aequorin gene (Prasher *et al.*, 1985, *Biochem. Biophys. Res. Comm.*, **126**:1259), which may be employed in calcium-sensitive bioluminescence detection; a green fluorescent protein gene (Niedz *et al.*, 1995 *Plant Cell Reports*, **14**:403); a luciferase (*luc*) gene (Ow *et al.*, 1986, *Science*, **234**:856), which allows for bioluminescence detection; a β-lactamase gene (Sutcliffe, 1978, *Proc. Natl. Acad. Sci. USA* **75**:3737), which encodes an enzyme for which various chromogenic substrates are known (e.g., PADAC, a chromogenic cephalosporin); an R-locus gene, encoding a product that regulates the production of anthocyanin pigments (red colour) in plant tissues (Dellaporta *et al.*, 1988, in *Chromosome Structure and Function*, pp. 263-282); an α-amylase gene (Ikuta *et al.*, 1990, *Biotech.*, **8**:241); a tyrosinase gene (Katz *et al.*,

1983, *J. Gen. Microbiol.*, **129**:2703) which encodes an enzyme capable of oxidising tyrosine to dopa and dopaquinone which in turn condenses to form the easily detectable compound melanin; or a *xylE* gene (Zukowsky *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* **80**:1101), which encodes a catechol dioxygenase that can convert chromogenic catechols.

7. ***Introduction of the nucleic acid construct into plant cells***

[0198] The sequences of the present invention can be used to transform or transfect any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained. Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e. monocot or dicot, targeted for transformation. It is recognised that the transformation protocols may be used for transfection or introduction of the oligonucleotide sequences to disrupt R gene function. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway *et al.*, 1986, *Biotechniques* 4:320-334), electroporation (Riggs et al., 1986, *Proc. Natl. Acad Sci. USA* **83**:5602-5606), Agrobacterium-mediated transformation (Townsend *et al.*, U.S. Pat. No. 5,563,055; Zhao *et al.*, U.S. Pat. No. 5,981,840), direct gene transfer (Paszkowski *et al.*, 1984, *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford et al., U.S. Pat. No. 4,945,050; Tomes *et al.*, U.S. Pat. No. 5,879,918; Tomes *et al.*, U.S. Pat. No. 5,886,244; Bidney et al., U.S. Pat. No. 5,932,782; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe *et al.*, (1988, *Biotechnology* 6:923-926). Also see Weissinger *et al.* (1988 *Ann. Rev. Genet.* **22**:421-477), Sanford *et al.*, (1987, *Particulate Science and Technology* **5**:27-37; onion), Christou *et al.*, (1988, *Plant Physiol.* **87**:671-674; soybean); Datta *et al.*, (1990, *Biotechnology* **8**:736-740; rice), Klein *et al.* (1988, *Proc. Natl. Acad. Sci. USA* 85:4305-4309, maize), Hooykaas-Van Slogteren *et al.* (1984, *Nature* (London) **311**:763-764; cereals), Bowen *et al.*, (U.S. Pat. No. 5,736,369; cereals), Bytebier *et al.*, (1987, *Proc. Natl. Acad. Sci. USA* **84**:5345-5349; Liliaceae), De Wet *et al.* (1985, in The Experimental Manipulation of Ovule Tissues, ed. Chapman *et al.* (Longman, N.Y.), pp. 197-209; pollen), Kaeppler *et al.*, (1990, *Plant Cell Reports* **9**:415-418; 1992, *Theor. Appl. Genet.* **84**:560-566; whisker-mediated transformation), D'Halluin *et al.* (1992, *Plant Cell* 4:1495-1505; electroporation); Li *et al.*, (1993, *Plant Cell Reports* **12**:250-255; rice), Christou and Ford (1995, *Annals of Botany* **75**:407-413; rice) and Osjoda *et al.* (1996, *Nature Biotechnology* **14**:745-750; maize *via* Agrobacterium tumefaciens). Guidance in the practical implementation of transformation systems for plant improvement is provided by Birch (1997, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* **48**: 297-326).

[0199] In certain embodiments, the present invention is concerned with transforming monocotyledonous plants, including graminaceous and non-graminaceous monocotyledonous plants. Illustrative examples of non-graminaceous monocotyledonous plants inlcude, but are not limited to,

Musaceae (*Musa* and *Ensete*), taro, ginger, onions, garlic, pineapple, bromeliaeds, palms, orchids, lilies, irises and the like. There are a variety of methods known currently for transformation of monocotyledonous plants. Presently, preferred methods for transformation of monocots are microprojectile bombardment of explants or suspension cells, and direct DNA uptake or electroporation as, for example, described by Shimamoto *et al.* (1989, *supra*). Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus bar* gene into embryogenic cells of a maize suspension culture by microprojectile bombardment (Gordon-Kamm, 1990, *Plant Cell*, 2:603-618). The introduction of genetic material into aleurone protoplasts of other monocotyledonous crops such as wheat and barley has been reported (Lee, 1989, *Plant Mol. Biol.* 13:21-30). Wheat plants have been regenerated from embryogenic suspension culture by selecting only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, 1990, *Bio/Technol.* 8:429-434). The combination with transformation systems for these crops enables the application of the present invention to monocots. These methods may also be applied for the transformation and regeneration of dicots. Transgenic sugarcane plants have been regenerated from embryogenic callus as, for example, described by Bower et al. (1996, *Molecular Breeding* 2:239-249).

8. Production and characterisation of differentiated transgenic plants

8.1 Regeneration

[0200] The methods used to regenerate transformed cells into differentiated plants are not critical to this invention, and any method suitable for a target plant can be employed. Normally, a plant cell is regenerated to obtain a whole plant following a transformation process.

[0201] Regeneration from protoplasts varies from species to species of plants, but generally a suspension of protoplasts is made first. In certain species, embryo formation can then be induced from the protoplast suspension, to the stage of ripening and germination as natural embryos. The culture media will generally contain various amino acids and hormones, necessary for growth and regeneration. Examples of hormones utilised include auxins and cytokinins. It is sometimes advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these variables are controlled, regeneration is reproducible. Regeneration also occurs from plant callus, explants, organs or parts. Transformation can be performed in the context of organ or plant part regeneration as, for example, described in *Methods in Enzymology*, Vol. 118 and Klee *et al.* (1987, *Annual Review of Plant Physiology*, 38:467), which are incorporated herein by reference. Utilising the leaf disk-transformation-regeneration method of Horsch *et al.* (1985, *Science*, 227:1229, incorporated herein by reference), disks are cultured on selective media, followed by shoot formation in about 2-4 weeks. Shoots that develop are excised from calli and transplanted to appropriate root-

inducing selective medium. Rooted plantlets are transplanted to soil as soon as possible after roots appear. The plantlets can be repotted as required, until reaching maturity.

[0202] In vegetatively propagated crops, the mature transgenic plants are propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection of desirable transgenotes is made and new varieties are obtained and propagated vegetatively for commercial use.

[0203] In seed propagated crops, the mature transgenic plants can be self-crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced foreign gene(s). These seeds can be grown to produce plants that would produce the selected phenotype, e.g., early flowering.

[0204] Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit, and the like are included in the invention, provided that these parts comprise cells that have been transformed as described. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced nucleic acid sequences.

[0205] The literature describes numerous techniques for regenerating specific plant types and more are continually becoming known. Those of ordinary skill in the art can refer to the literature for details and select suitable techniques without undue experimentation.

8.2 Characterisation

[0206] To confirm the presence of a R polynucleotide of the invention in the regenerating plants, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting and PCR; an R protein expressed by the polynucleotide of the invention may be assayed using antigen-binding molecules as for example described herein.

[0207] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting example.

EXAMPLES

EXAMPLE 1

Identification of R genes from *M. acuminata*

CTAB total DNA purification

[0208] Genomic DNA was extracted using the CTAB protocol of Stewart & Via (1993). Briefly, leaf tissue was frozen in liquid N_2 and ground in a mortar and pestle. Powdered tissue was resuspended in CTAB Buffer (1% Sarcosine, 0.8 M NaCl, 0.022 M EDTA pH8.0, 0.22 M Tris-HCl pH 7.8, 0.8% CTAB, 0.14 M Mannitol) at 65°C. An equal volume of chloroform:isoamyalcohol (24:1) was immediately added, mixed by inversion and incubated at 65°C for 10 min with occasional inversion. Samples were centrifuged for 5 min at 14000 rpm in a microfuge to separate phases. The aqueous layer was collected and an equal volume of isopropanol added. DNA was spooled out, washed in 70% ethanol, and allowed to dry before resuspending in 100 µL dH_2O containing RNaseA (1 mg/mL).

Purification of total RNA

[0209] Total RNA extractions were performed using the method of Chang *et al.* (1993). Tissue was frozen in liquid N_2 and ground to a powder in a mortar and pestle. Powdered tissue was added to preheated (65° C) extraction buffer (2% CTAB, 2% PVP, 100 mM Tris HCl pH8, 25 mM EDTA, 2 M NaCl, 0.05% spermidine, 2% beta-mercaptoethanol). Chloroform:isoamylalcohol (24:1) was added, the suspension vortexed, and samples centrifuged at top speed in a microfuge for 5 min. The aqueous phase was collected and an equal volume of DEPC-treated 4M LiCl added. RNA was precipitated overnight 4° C and then centrifuged at 4° C for 30 min at top speed. Pelleted RNA was resuspended in 10 X SSTE and extracted once more with chloroform:isoamylalcohol (24:1). The RNA was reprecipitated at –20° C overnight following the addition of 1/10 volume DEPC-treated 2.5 M NaOAc pH6.0 and 21/2 volumes of 100% ethanol. Tubes were centrifuged 20 min, the pellets washed with 70% ethanol and resuspended in DEPC-treated dH_2O.

Reverse-transcriptase PCR of banana R-genes

[0210] Sequences of R-genes from plant species were aligned and degenerate primers designed to conserved motifs in the NBS regions. The degenerate primers were used to generate single-stranded cDNAs from total RNA using reverse transcriptase and then to subsequently amplify the NBS region of the banana R-genes. To generate the region 5' of the NBS domain, RNA primers were ligated to the 5' end of the mRNA after removal of the 5'-cap structure. Ligated mRNA was reverse transcribed using reverse transcriptase to generate single-stranded cDNA. Primer complementary to the ligated RNA primer and a specific primer to the known NBS sequence was added and PCR undertaken to

generate the 5' region of the R-gene using the parameters of: initial denaturation step of 94° C for 2 min followed by 5 cycles of 94° C for 30 secs, 55-65° C for 30 secs, 72° C for 3-5 min, then 25 cycles of 94° C for 30 secs, 45-60° C for 30 secs, 72° C for 3-5 min, followed by a final annealing step 72° C for 10 min. N-terminal and C-terminal primers were subsequently used to amplify complete R-gene sequences from genomic DNA using PCR with the following conditions: initial denaturation step of 94° C for 2 min, followed by 25 cycles of 94° C for 30 secs, 55° C for 30 secs, 72° C for 1-5 min, followed by a final annealing step 72° C for 10 min. All PCR products were cloned and sequenced to verify identity. The full-length nucleotide sequences for two R genes, one isolated from *Musa acuminata* (Calcutta 4) designated RGA5 and the other from *Musa acuminata* spp *malaccensis* designated RGA2, are presented in SEQ ID NO: 1 and 3, respectively.

[0211] RT-PCR was then used to compare the expression of the R genes between *M. acuminata* spp *malaccensis* plants that were susceptible or resistant to *Fusarium oxysporum* fsp *cubense* (FOC). The results presented in Figure 3 show that the RGA2 gene (see lanes C2) is transcribed in FOC resistant plants but not in FOC sensitive plants. This suggests that RGA2 may be an attractive candidate for conferring disease resistance to susceptible plants. The inventors propose to transform Cavendish, which is resistant to race 1 but susceptible to race 4, (i) with RGA2 only; (ii) with RGA5 and (iii) with both RGA2 and RGA5, under the control of a heterologous promoter (e.g., Ubi) or the native RGA2 promoter.

[0212] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0213] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0214] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

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